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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
MOUSE MONOCLONAL
AB-T17

Example of ELISA protocol used to test conjugated L-DOPA:

1. Coating of conjugated L-DOPA (15 µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05 M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001 M, for sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5 g/l of BSA (Acros), 0.05% Tween 20 (Acros) and SMB 0.001 M for one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated L-DOPA antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5 g/l BSA, 10% of glycerol and SMB 0.001 M, 200 µl by well plate (incubating for 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 µl of peroxidase-labeled goat anti-mouse IgG (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5 g/l BSA, 10% of glycerol, 0.5% Tween and SMB 0.001 M, will be applied by well plate (for one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200 µl by well plate of a citrate 0.1M/phosphate, 0.2 M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, stop the reaction by the addition of 50 µl of 2 M HCl.
9. The optical density will be measured at 492 nm, to obtain the different values (IC 50).

ELISA Protocol:

1. Coat microtitration plates with 10 µg of conjugated GSH-G-BSA per ml of carbonate buffer (pH 9.6). Agitate overnight at 4°C. Plate 200 µl per well.
2. Block 1 hour at 37°C with PBS Tween buffer + 10% Glycerol + 1 g/L BSA.
3. Rinse 3 times with PBS Tween.
4. Primary Antibody: Dilute serum 1/10,000 in PBS Tween buffer + 10% Glycerol + 1 g/L of BSA + 1 g/L of BSA-G. Incubate 2 hours at 37°C.
5. Rinse 3 times with PBS Tween.
6. Secondary Antibody: Dilute peroxidase-conjugated anti-Rabbit IgG to 1/10,000 in PBS Tween buffer + 1 g/L of BSA. Incubate 1 hour at 37°C.
7. Rinse 3 times with PBS Tween.
8. Development: Add 20 µl H_2O_2 and 1 ml OPD 4% in 20 ml of citrate phosphate buffer. Plate 200 µl per well. Put the well-plates in the dark for 10 minutes. Stop the reaction with 50 µl HCl, 2N.
9. Read the optical density at 492 nm.

G = Glutaraldehyde, BSA = Bovine Serum Albumin, GSH = Glutathione

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Anti-Conjugated Dopamine
MOUSE MONOCLONAL
AB-T11

Example of ELISA protocol used to test conjugated dopamine:

1. Coating of conjugated dopamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated dopamine antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M , 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-mouse IgG (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated dopamine:

Detection of conjugated Dopamine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   Solution A (30ml): 150-300ml/min
   Solution B (500ml): 150-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Dopamine antibody: The final dilution is 1/1,000 to 1/5,000 in solution C containing 0.1% triton X100, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: The antibody may be used at a higher dilution. The customer should explore the further antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/200 dilution of goat anti-mouse in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of mouse peroxidase/anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H2O2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated Dopamine
MOUSE MONOCLONAL
AB-T11

Example of Immunohistochemistry used to test conjugated Tryptamine and dopamine:

Simultaneous detection of Tryptamine and Dopamine in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 0.5M glutaraldehyde (G) solution in 0.1M cacodylate buffer pH 7.5 containing 0.9% sodium metabisulfite (SMB).
2. Post fixation: The brain will be removed, post-fixed for 60min in 0.5M glutaraldehyde (G) solution containing 0.9% sodium metabisulfite (SMB) and then washed thoroughly with 0.05M Tris buffer containing 0.05M SMB (Tris-SMB) pH 7.5.
3. Tissue sectioning: Vibratome sections will be cut through the region from the substantia to the raphe nuclei for the molecular detection of dopamine (DA) and tryptamine (T) .
4. Application of anti-conjugated antiserum: Sections will be placed in Tris-SMB buffer, reduced using 0.1M sodium borohydride (in the same buffer), washed and then processed for immunocytochemistry using the peroxide / anti-peroxide (PAP) method. Following incubation with 3% non-specific serum in Tris-SMB for 1h at room temperature, the sections are incubated overnight at 4°C in Tris-SMB buffer containing 1% non specific serum, 0.02% Triton X100, diluted antibodies against DA and T (final dilutions 1/1,000 to 1/5,000). The T antiserum had previously been purified on the glutaraldehyde-conjugated protein carriers used during immunization and a monoclonal antibody to conjugated DA was produced.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: After rinsing with Tris-NaCl buffer (0.05M Tris containing 0.9% NaCl pH 7.4), sections will be incubated at 37°C in 1/100 dilutions of goat anti-mouse IgG antibodies and swine anti-rabbit IgG antibodies (DAKO) .
   PAP / Revelation: After another wash in Tris-NaCl buffer, sections will be incubated (1 hour at 37°C) with a 1/500 dilution of mouse PAP complex. The sections will be then washed again, and the reaction is developed in 0.05% 3-3'-diaminobenzidine (DAB) containing 0.01% hydrogene peroxide.
   After thorough washing in Tris-NaCl buffer, the sections will be incubated with a 1/1,000 dilution of rabbit PAP complex for 1 hour at 37°C, washed again and developed in a mixture of 0.025% DAB and 0.3% nickel ammonium sulphate containing 0.01% hydrogene peroxide. The sections will be mounted on slides using phosphate-buffered saline (PBS/glycerol, 1:3 v/v), placed under coverclips and observed under transmitted bright-field illumination.
   Control sections will be prepared either by replacing one or two specific antibodies with non-immune rabbit or mouse serum, or by sequentially omitting each step of the immunological procedure.
   To determine the specificity of each staining, the antibodies against DA and T will be incubated overnight at 4°C with each of the following conjugates: dopamine-glutaraldehyde-protein carrier (DA-G-Pc), HT-G-Pc, MT-G-Pc and T-G-Pc. Each mixture is then centrifuged at 10,000g for 15min, and the supernatants is used for the primary incubation in the immunocytochemical procedure described above. In these adsorption tests, the final dilutions of the antibodies against DA and T are identical to those used with the non-adsorbed antibodies. The final concentrations of each conjugated neurotransmitter (DA or HT, MT, T) is from 10-8M to 10-9M. Some sections were treated by the single peroxidase / antiperoxidase method. Thus, we compared both dopamine-T-Immunoreactivity (DA-IR) and T-IR in the same rat brain regions.

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Anti-Conjugated Acetylcholine
MOUSE MONOCLONAL
AB-T029

Example of ELISA protocol used to test conjugated choline-glutaric acid:

1. Coating of conjugated choline-glutaric acid (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated choline-glutaric acid antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-mouse (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37° C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated choline-glutaric acid:

Detection of conjugated choline-glutaric acid in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following fixative solution: glutaraldehyde 0.5M, 2-nitrobenzyl alcohol 0.1M, sodium metabisulfite10g/l and cacodylate 0.1M, sodium bromide 0.01M, pH 10.7 (solution A: 200-300ml/min).
2. Post fixation: 2h in 0.5M glutaraldehyde solution (pH 7.5) without the 2-nitrobenzyl alcohol, then 4 soft washes in Tris 0.05M with sodium metabisulfite 10g/l, pH 7.4 (solution B).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
   The sections were washed 4 times in solution B, and incubated for 1h at 37°C in solution B containing 0.2% triton X100, plus 1% of non specific serum.
4. Application of anti-conjugated Acetylcholine antibody: The final dilution is 1/1,000 to 1/5,000 in solution B containing 0.2% triton X100, plus 1% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution B.

Note: The antibody may be used at a higher dilution. The customer should explore the further antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/200 dilution of goat anti-mouse antibodies diluted at 1/100 in solution B for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution B;
   PAP: Sections are incubated with mouse peroxidase/anti-peroxidase complex diluted at 1/500 in solution B for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution B;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.3% of nickel ammonium sulfate; 0.05% of H2O2 were added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Glutamate
MOUSE MONOCLONAL
AB-T12

Example of ELISA protocol used to test conjugated L-glutamate:

1. Coating of conjugated L-glutamate (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated L-glutamate antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-mouse IgG (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated L-glutamate:

Detection of conjugated Glutamate in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 150-300ml/min
   solution B (500ml): 150-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Glutamate antibody: The final dilution is 1/1,000 to 1/5,000 in solution C containing 0.1% triton X100, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of monoclonal antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: The antibody may be used at a higher dilution. The customer should explore the further antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/200 dilution of goat anti-mouse in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of mouse peroxidase/anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Glutamate
MOUSE MONOCLONAL
AB-T12

Example of Immunohistochemistry used to test conjugated Glutamate:

Detection of conjugated Glutamate in cockroach brain

1. Fixation: Cockroach brains were fixed overnight at 4°C in fixative comprising 1% glutaraldehyde, 2.5% paraformaldehyde, and 1% sodium metabisulfite (SMB, Sigma) in 0.1M cacodylate buffer adjusted to pH 7.2.
2. After fixation, whole brains were immersed in 10-2 M sodium borohydride ($NaBH_4$, Sigma) in a solution of 0.05M Tris-HCl buffer with 0.5% SMB pH 7.5 (Chagnaud et al., 1989).
3. Tissue sectioning: After a wash in 0.05M Tris-HCl-SMB buffer, brains were embedded in 8% agarose for serial 80μm frontal and sagittal sections.
4. Application of anti-conjugated antiserum: Sections were incubated with 10% normal swine serum in 0.05M Tris-HCl-SMB with 0.5% TritonX100 (Tx).
   Application of anti-conjugated rabbit glutamate antiserum: Sections were incubated overnight at room temperature in rabbit glutamate antiserum diluted to 1/1000-1/5000.
5. Revelation:
   Second antibody: After a wash in Tris-HCl-Tx, sections were incubated overnight with goat anti-rabbit immunoglobulin conjugated to Texas Red (1/250 Tris-HCl-Tx, Jackson Laboratories). After a final wash in Tris-HCl, the sections were embedded in the 80% glycerol.

   To double label glutamate and taurine, agarose sections were incubated overnight with mouse monoclonal anti-glutamate antibodies at a dilution of 1/100 together with rabbit polyclonal anti-taurine antibodies (1/500) in Tris-HCl-Tx. After washing, the secondary antibodies goat anti-rabbit immunoglobulin conjugated to Texas Red (1/250) and Alexa 488 goat anti-mouse immunoglobulin conjugate (1/250) were applied simultaneously to the sections for incubation at room temperature overnight. After a 6-8 hours wash, sections were mounted in glycerol.

   Using other antibodies raised in rabbit and this monoclonal, double labeling can be done.

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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
MOUSE MONOCLONAL
AB-T17

Example of Immunohistochemistry used to test conjugated L-DOPA conjugate:

Detection of conjugated L-DOPA in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 100ml 0.1M cacodylate buffer containing 5% glutaraldehyde (G) and 0.9% sodium metabisulfite (SMB) pH 7.5.
2. Post fixation: Brains will be removed quickly and post-fixed for 2 hours at 4°C in the same solution.
3. Tissue sectioning: Serial transverse vibratome sections will be cut in Tris- SMB.
4. Application of anti-conjugated antiserum: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB pH 7.5).
   Then, the sections will be washed with in the same solution and incubated in Tris-SMB containing 3% non-specific serum (1h at 4°C):
   Application of anti-conjugated L-DOPA antibodies: The sections will be incubated floating free in plastic wells containing rabbit conjugated L-DOPA antiserum (1/,1000 to 1/5,000) or monoclonal anti conjugated DA antibody (1/,1000 to 1/5,000) for 2 days at 4°C in Tris-SMB buffer pH 7.4.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: After rinsing 3 times for 10 min in 0.1M Tris buffer containing 0.9% NaCl (Tris-NaCl) pH 7.4, the sections will be incubated with swine anti-rabbit IgG antibodies (Dako) or goat anti-mouse IgG antibodies (Dako). Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum.
   PAP: Rinsed again, sections will then be incubated (1 hour at 37°C) in Tris-NaCl with a 1/500 dilution of rabbit or mouse peroxidase anti-peroxidase (PAP) complex (Dako).
   Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3'diaminobenzidine (DAB ,Sigma) and 0.01% hydrogen peroxide (Merck).
   The reaction will be stopped by the transfer of the sections in Tris-NaCl buffer.

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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
MOUSE MONOCLONAL
AB-T17

Example of Immunohistochemistry used to test conjugated L-DOPA conjugate:

Double detection of conjugated L-DOPA and Dopamine (DA) in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 5% glutaraldehyde (G), 0.9% sodium metabisulfite (SMB) solution in 0.1cacodylate buffer pH 7.4.
2. Post fixation: 2H, 4°C in the same fixative solution.
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated antiserum: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB).
   Then, the sections will be washed in the same solution (12h, 4°C) and incubated in Tris-SMB containing 3% non specific serum and 0.1% Triton X100 (8h at 4°C).
   Application of anti-conjugated L-DOPA antibodies: Free Floating adjacent sections will be incubated (24h, 4°C) with a monoclonal antibody against G-conjugated L-DOPA (1/,1000 to 1/5,000), with a monoclonal antibody against G-conjugated DA (1/,1000 to 1/5,000), and with both. Antibodies will be diluted in Tris-SMB, 1% non-specific serum, 0.2% Triton X100 solution.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: After rinsing, sections will be incubated (12 hours at 4°C) with respectively swine anti-rabbit IgG antibodies (Dako), goat anti-mouse IgG antibodies (Dako) and both. Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum.
   PAP: Rinsed again, sections will then be incubated (1 hour at 37°C) with a 1/1,000 dilution of rabbit peroxidase anti-peroxidase (PAP) complex (Dako) for single L-DOPA detection and 1/500 dilution of PAP mouse complex for DA detection.
   Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3'diaminobenzidine (DAB ,Sigma) plus cobalt chloride (Sigma, 10mg/20ml) and 0.01% hydrogene peroxide (30vol., Merck).

   You must repeat the protocol; do step of PAP and develop with DAB after the second PAP with DADNi.

   For the double detection of L-DOPA and DA, the sections which have received anti-DA together with L-DOPA antibodies and then anti-rabbit together with anti-mouse secondary antibodies, will be incubated with PAP mouse complex, then revealed in DAB plus cobalt chloride, giving a dark-blue color. These staining sections will be then washed thoroughly (12 hours at 4°C) and incubated with PAP rabbit complex, then revealed in a 0.1% DAB solution giving a yellow-brown coloration.

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Anti-Conjugated Histamine
MOUSE MONOCLONAL
AB-T16

Example of ELISA protocol used to test conjugated histamine:

1. Coating of conjugated histamine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated histamine antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-mouse IgG (Biorad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of ELISA protocol used to test conjugated 5-hydroxytryptamine:

1. Coating of conjugated 5-hydroxytryptamine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-hydroxytryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated 5-Hydroxytryptamine (Serotonin)
RABBIT POLYCLONAL
AB-T03

Example of Immunohistochemistry used to test conjugated 5-hydroxytryptamine:

Detection of conjugated 5-hydroxytryptamine (serotonin) in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated 5-hydroxytryptamine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

Example of ELISA protocol used to test conjugated tryptamine:

1. Coating of conjugated tryptamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M , during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol ,0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated Tryptamine
RABBIT POLYCLONAL
AB-T04

Example of Immunohistochemistry used to test conjugated tryptamine:

Detection of conjugated Tryptamine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   olution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Tryptamine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated Tryptamine
RABBIT POLYCLONAL
AB-T04

Example of Immunohistochemistry used to test conjugated Tryptamine and Dopamine:

Simultaneous detection of Tryptamine and Dopamine in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 0.5M glutaraldehyde (G) solution in 0.1M cacodylate buffer pH 7.5 containing 0.9% sodium metabisulfite (SMB).
2. Post fixation: The brain will be removed , post-fixed for 60min in 0.5M glutaraldehyde (G) solution containing 0.9% sodium metabisulfite (SMB) and then washed thoroughly with 0.05M Tris buffer containing 0.05M SMB (Tris-SMB) pH 7.5.
3. Tissue sectioning: Vibratome sections will be cut through the region from the substantia to the raphe nuclei for the molecular detection of dopamine (DA) and tryptamine (T) .
4. Reduction step: Sections will be placed in Tris-SMB buffer, reduced using 0.1M sodium borohydride (in the same buffer), washed and then processed for immunocyto-chemistry using the peroxide / anti-peroxide (PAP) method. Following incubation with 3% non-specific serum in Tris-SMB for 1h at room temperature, the sections are incubated overnight at 4°C in Tris-SMB buffer containing 1% non specific serum, 0.02% Triton X100, diluted antibodies against DA and T (final dilutions 1/1,000 to 1/5,000). The T antiserum had previously been purified on the glutaraldehyde-conjugated protein carriers used during immunization and a monoclonal antibody to conjugated DA was produced.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: After rinsing with Tris-NaCl buffer (0.05M Tris containing 0.9% NaCl pH 7.4), sections will be incubated at 37°C in 1/100 dilutions of goat anti-mouse IgG antibodies and swine anti-rabbit IgG antibodies (DAKO) .
   PAP / Revelation: After another wash in Tris-NaCl buffer, sections will be incubated (1 hour at 37°C) with a 1/500 dilution of mouse PAP complex. The sections will be then washed again, and the reaction is developed in 0.05% 3-3'-diaminobenzidine (DAB) containing 0.01% hydrogene peroxide.
   After thorough washing in Tris-NaCl buffer, the sections will be incubated with a 1/1,000 dilution of rabbit PAP complex for 1 hour at 37°C, washed again and developed in a mixture of 0.025% DAB and 0.3% nickel ammonium sulphate containing 0.01% hydrogene peroxide. The sections will be mounted on slides using phosphate-buffered saline (PBS/glycerol, 1:3 v/v), placed under coverclips and observed under transmitted bright-field illumination.
   Control sections will be prepared either by replacing one or the two the specific antibodies with non-immune rabbit or mouse serum, or by sequentially omitting each step of the immunological procedure.
   To determine the specificity of each of staining, the antibodies against DA and T will be incubated overnight at 4°C with each of the following conjugates: dopamine-glutaraldehyde-protein carrier (DA-G-Pc), HT-G-Pc, MT-G-Pc and T-G-Pc. Each mixture is then centrifuged at 10,000g for 15min, and the supernatants is used for the primary incubation in the immunocytochemical procedure described above. In these adsorption tests, the final dilutions of the antibodies against DA and T are identical to those used with the non-adsorbed antibodies. The final concentrations of each conjugated neurotransmitter (DA or HT, MT, T) is from 10-8 M to 10-9 M. Some sections were treated by the single peroxidase / antiperoxidase method. Thus, we compared both dopamine-T-Immunoreactivity (DA-IR) and T-IR in the same rat brain regions.

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Anti-Conjugated Noradrenaline
RABBIT POLYCLONAL
AB-T06

Example of ELISA protocol used to test conjugated noradrenaline:

1. Coating of conjugated noradrenaline (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated noradrenaline antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA,10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0,2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated noradrenaline:

Detection of conjugated Noradrenaline in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Noradrenaline antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated Dopamine
RABBIT POLYCLONAL
AB-T07

Example of ELISA protocol used to test conjugated dopamine conjugate:

1. Coating of conjugated dopamine conjugate (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M (Acros), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated dopamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated dopamine conjugate:

Detection of conjugated Dopamine in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
   Application of anti-conjugated Dopamine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.1%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated Dopamine
RABBIT POLYCLONAL
AB-T07

Example of Immunohistochemistry used to test conjugated Tryptamine and dopamine

Simultaneous detection of Tryptamine and Dopamine (DA) in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 0.5M glutaraldehyde (G) solution in 0.1M cacodylate buffer pH 7.5 containing 0.9% sodium metabisulfite (SMB).
2. Post fixation: The brain will be removed , post-fixed for 60min in 0.5M glutaraldehyde (G) solution containing 0.9% sodium metabisulfite (SMB) and then washed thoroughly with 0.05M Tris buffer containing 0.05M SMB (Tris-SMB) pH 7.5.
3. Tissue sectioning: Vibratome sections will be cut through the region from the substantia to the raphe nuclei for the molecular detection of dopamine (DA) and tryptamine (T) .
4. Reduction step: Sections will be placed in Tris-SMB buffer, reduced using 0.1M sodium borohydride (in the same buffer), washed and then processed for immunocyto-chemistry using the peroxide / anti-peroxide (PAP) method.
5. Primary antibody: Following incubation with 3% non-specific serum in Tris-SMB for 1h at room temperature, the sections are incubated overnight at 4°C in Tris-SMB buffer containing 1% non specific serum, 0.02% Triton X100, diluted antibodies against DA and T (final dilutions 1/10,000). The T antiserum had previously been purified on the glutaraldehyde-conjugated protein carriers used during immunization and a monoclonal antibody to conjugated DA was produced.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: After rinsing with Tris-NaCl buffer (0.05M Tris containing 0. 9% NaCl pH 7.4), sections will be incubated at 37°C in 1/100 dilutions of goat anti-mouse IgG antibodies and swine anti-rabbit IgG antibodies (DAKO) .
   PAP / Revelation: After another wash in Tris-NaCl buffer, sections will be incubated (1 hour at 37°C) with a 1/500 dilution of mouse PAP complex. The sections will be then washed again, and the reaction is developed in 0.05% 3-3'-diaminobenzidine (DAB) containing 0.01% hydrogene peroxide.
   After through washing in Tris-NaCl buffer, the sections will be incubated with a 1/1,000 dilution of rabbit PAP complex for 1 hour at 37°C, washed again and developed in a mixture of 0.025% DAB and 0.3% nickel ammonium sulphate containing 0.01% hydrogene peroxide. The sections will be mounted on slides using phosphate-buffered saline (PBS/glycerol, 1:3 v/v), placed under coverclips and observed under transmitted bright-field illumination.

   You must repeat the protocol; do step of PAP and develop with DAB after the second PAP with DADNi.

   Control sections will be prepared either by replacing one or the two specific antibodies with non-immune rabbit or mouse serum, or by sequentially omitting each step of the immunological procedure.
   To determine the specificity of each of staining, the antibodies against DA and T will be incubated overnight at 4°C with each of the following conjugates: dopamine-glutaraldehyde-protein carrier (DA-G-Pc), HT-G-Pc, MT-G-Pc and T-G-Pc. Each mixture is then centrifuged at 10,000g for 15min, and the supernatants is used for the primary incubation in the immunocytochemical procedure described above. In these adsorption tests, the final dilutions of the antibodies against DA and T are identical to those used with the non-adsorbed antibodies. The final concentrations of each conjugated neurotransmitter (DA or HT, MT, T) is from 10-8 M to 10-9 M. Some sections were treated by the single peroxidase / antiperoxidase method. Thus, we compared both dopamine-T-Immunoreactivity (DA-IR) and T-IR in the same rat brain regions.

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Anti-Conjugated L-Glutamate
RABBIT POLYCLONAL
AB-T08

Example of ELISA protocol used to test conjugated L-glutamate:

1. Coating of conjugated L-glutamate (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated L-glutamate antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Immunohistochemistry used to test conjugated L-glutamate:

Detection of conjugated Glutamate in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated Glutamate antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated 5-Hydroxytryptophan
RABBIT POLYCLONAL
AB-T09

Example of ELISA protocol used to test conjugated 5-hydroxytryptrytophan:

1. Coating of conjugated 5-hydroxytryptophan (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite 0.001M (SMB), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-hydroxytryptophan antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated 5-hydroxytryptrytophan:

Detection of conjugated 5-hydroxytryptophan in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5% pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated 5-hydroxytryptophan antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

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Anti-Conjugated GABA (Gamma-Aminobutyric acid)
RABBIT POLYCLONAL
AB-T10

Example of ELISA protocol used to test conjugated GABA:

1. Coating of conjugated GABA (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated GABA antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Immunohistochemistry used to test conjugated GABA:

Detection of conjugated GABA in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%; pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated GABA antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H2O2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Aspartate
RABBIT POLYCLONAL
AB-T022

Example of ELISA protocol used to test conjugated L-Aspartatic acid:

1. Coating of conjugated L-Aspartatic acid (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated L-Aspartatic acid antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Immunohistochemistry used to test conjugated L-Aspartatic acid:

Detection of conjugated L-Aspartatic acid in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated L-Aspartatic acid antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Aspartate
RABBIT POLYCLONAL
AB-T022

Example of immunohistochemistry used to test conjugated L-Aspartate and Taurine:

Detection of conjugated Aspartate and Taurine in cockroach brain

1. Post fixation: For aspartate and taurine immunostaining, brains will be fixed overnight in 4% paraformaldehyde / 1% glutaraldehyde in 0.1M phosphate buffer (pH 7.3).
2. Tissue sectioning: Brains will be embedded in 7-8% agarose or gelatine/albumin and sectioned at 60-100 μm with a vibratome (Leica).
3. Application of anti-conjugated antiserum: After washing with phosphate buffer containing 0.3% TritonX100 (PBST), sections will be incubated in the same buffer with 10% normal swine serum (Dako Corp.).
   Application of anti-conjugated Aspartate antiserum or Taurine antiserum: Sections will be then incubated overnight with aspartate antiserum (1/1,000) or taurine antiserum (1/1,000) at room temperature.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

4. Revelation: Second antibody: After a PBST wash, secondary antibodies will be applied overnight. These consisted of goat anti-rabbit immunoglobulins conjugated to Texas Red (1/250, Jackson ImmunoResearch Laboratories) or anti-rabbit immunoglobulins conjugated to Alexa 568 (Molecular probes).
   After a final wash, sections will be mounted on slides and cover–slipped under 80% glycerol.

An immunofluorescence double staining method will be used to simultaneously reveal taurine and aspartate immunoreactivity in the gelatin sections. This entailed two sequences of primary and secondary labeling.

1. Aspartate antiserum (1/1,000) will be applied to gelatin sections overnight.
2. After a 6-hour wash, sections will be exposed for 12-18 hours to biotinylated swine anti-rabbit immunoglobulins diluted to 1/250 (Dako Corp.) in PBST.
3. Sections will be next washed for 6 hours in 0.01M PBST. This step will be followed by an overnight incubation in streptavidin-fluorescein (1/100, Jackson ImmunoResearch Laboratories). The above concentration of biotinylated swine anti-rabbit immunoglobulins blocked all antigen sites of the primary antibody.
4. After completing this stage, sections will be washed for 6-8 hours and then incubated overnight with rabbit taurine antiserum (1/500).
5. After a 6 hour wash in PBST, the sections will be incubated overnight with goat anti-rabbit immunoglobulins conjugated to Texas Red (1/250) or Cy5 (1/250). As a control, PBST replaced the aspartate antiserum and the taurine antiserum. No interaction between the reagents of the first and second layers of antibodies will be observed.

Example of ELISA protocol used to test conjugated glycine:

1. Coating of conjugated glycine (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated glycine antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Glycine
RABBIT POLYCLONAL
AB-T023

Example of Immunohistochemistry used to test conjugated glycine:

Detection of conjugated Glycine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Glycine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

Example of ELISA procol used to test conjugated tyrosine:

1. Coating of conjugated tyrosine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tyrosine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated Tyrosine
RAT POLYCLONAL
AB-T031

Example of ELISA protocol used to test conjugated tyrosine:

1. Coating of conjugated tyrosine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tyrosine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of ELISA protocol used to test conjugated L-cysteine:

1. Coating of conjugated L-cysteine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated L-cysteine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated L-Cysteine
RABBIT POLYCLONAL
AB-T034

Example of immunohistochemistry used to test conjugated L-cysteine:

Perfusion Protocol for Adult Male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing L-cysteine antiserum (diluted as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated D-Glutamic Acid (D-Glutamate)
RABBIT POLYCLONAL
AB-T045

Example of ELISA protocol used to test conjugated D-Glutamate:

1. Coating of conjugated D-Glutamate (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated D-Glutamate antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated D-Glutamic Acid (D-Glutamate)
RABBIT POLYCLONAL
AB-T045

Example of Immunohistochemistry used to test conjugated D-Glutamate:

Perfusion Protocol for adult male Sprague-Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing D-Glutamic acid antiserum (diluted 1/1,000-1/5,000; as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated D-Aspartic Acid (D-Aspartate)
RABBIT POLYCLONAL
AB-T046

Example of ELISA protocol used to test conjugated D-Aspartatic acid:

1. Coating of conjugated D-Aspartatic acid (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated D-Aspartatic acid antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated D-Aspartic Acid (D-Aspartate)
RABBIT POLYCLONAL
AB-T046

Example of Immunohistochemistry used to test conjugated D-Aspartatic acid:

Perfusion protocol for adult male Sprague-Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized for example with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2, (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing D-Aspartic acid antiserum (diluted 1/1,000-1/5,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Pseudomonas fluorescens
RABBIT POLYCLONAL
AB-T063

Example of ELISA protocol used to test *Pseudomonas fluorescens*:

1. Coating of Pseudomonas fluorescens (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas fluorescens serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas fluorescens*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Pantoea agglomerans (Erwinia herbicola)
RABBIT POLYCLONAL
AB-T064

Example of ELISA protocol used to test conjugated *Pantoea agglomerans*:

1. Coating of Pantoea agglomerans (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pantoea agglomerans serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pantoea agglomerans*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted 1/1,000-1/2,000 in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200 ml) + (50 mg DAB in 25 ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Stenotrophomonas maltophilia
RABBIT POLYCLONAL
AB-T065

Example of ELISA protocol used to test *Stenotrophomonas maltophilia*:

1. Coating of Stenotrophomonas maltophilia (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Stenotrophomonas maltophilia serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/5,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Stenotrophomonas maltophilia*:

Membrane Blocking, Antibody Incubations and Detection of Proteins:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Pseudomonas stutzeri
RABBIT POLYCLONAL
AB-T066

Example of ELISA protocol used to test conjugated *Pseudomonas stutzeri*:

1. Coating of Pseudomonas stutzeri (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas stutzeri serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/5,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test conjugated *Pseudomonas stutzeri*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
RABBIT POLYCLONAL
AB-T067

Example of ELISA protocol used to test conjugated L-dihydroxyphenylalanine:

1. Coating of conjugated L-dihydroxyphenylalanine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Preabsorbed L-dihydroxyphenylalanine antiserum will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated L-dihydroxyphenylalanine:

Detection of conjugated L-DOPA in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated L-DOPA antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Dihydroxyphenylalanine (L-DOPA)
RABBIT POLYCLONAL
AB-T067

Example of Immunohistochemistry used to test conjugated L-dihydroxyphenylalanine:

Double detection of conjugated L-DOPA and Dopamine in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 5% glutaraldehyde (G), 0.9% sodium metabisulfite (SMB) solution in 0.1cacodylate buffer pH 7,4.
2. Post fixation: 2h, 4°C in the same fixative solution.
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Application of anti-conjugated antiserum: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB). Then, the sections will be washed in the same solution (12h, 4°C) and incubated in Tris-SMB containing 3% non specific serum and 0.1% Triton X100 (8h at 4°C).
5. Application of anti-conjugated L-DOPA antibodies: Free floating adjacent sections will be incubated (24h, 4°C) with a polyclonal antiserum against conjugated L-DOPA (1/,1000 to 1/5,000), with a monoclonal antibody against conjugated DA (1/,1000 to 1/5,000), and with both. Antisera will be diluted in Tris-SMB, 1% non-specific serum, 0.2% Triton X100 solution.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: After rinsing, sections will be incubated (12 hours at 4°C) with respectively swine anti-rabbit IgG antibodies (DAKO), goat anti-mouse IgG antibodies (DAKO) and both. Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum .
   PAP: Rinsed again, sections will be then incubated (1 hour at 37°C) with a 1/1,000 dilution of rabbit peroxidase anti-peroxidase (PAP) complex (DAKO) for single L-DOPA detection and 1/500 dilution of PAP mouse complex for DA detection.
   Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3'-diaminobenzidine (DAB ,Sigma) plus cobalt chloride (Sigma, 10mg/20ml) and 0.01% hydrogene peroxide (30vol., Merck).

   You must repeat the protocol: it needs that you must do step of PAP and develop with DAB after the second PAP with DADNi.

   For the double detection of L-DOPA and DA, the sections which have received anti-DA together with L-DOPA antibodies and then anti-rabbit together with anti-mouse secondary antibodies, will be incubated with PAP mouse complex, then revealed in DAB plus cobalt chloride, giving a dark-blue color. These staining sections will be then washed thoroughly (12 hours at 4°C) and incubated with PAP rabbit complex, then revealed in a 0.1% DAB solution giving a yellow-brown coloration.

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Anti-Conjugated Dopamine
RAT POLYCLONAL
AB-T068

Example of ELISA protocol used to test conjugated dopamine:

1. Coating of conjugated dopamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated dopamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA,10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated dopamine:

Detection of conjugated Dopamine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 150-300ml/min
   solution B (500ml): 150-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Dopamine antibody: The final dilution is 1/1,000 to 1/5,000 in solution C containing 0,1% triton X100, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: The antibody may be used at a higher dilution. The customer should explore the further antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/200 dilution of goat anti-mouse in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of mouse peroxidase/anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated Dopamine
RAT POLYCLONAL
AB-T068

Example of Immunohistochemistry used to test conjugated Tryptamine and Dopamine:

Simultaneous detection of Tryptamine and Dopamine in rat brain

1. Perfusion: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 0.5M glutaraldehyde (G) solution in 0.1M cacodylate buffer pH 7.5 containing 0.9% sodium metabisulfite (SMB).
2. Post fixation: The brain will be removed, post-fixed for 60min in 0.5M glutaraldehyde (G) solution containing 0.9% sodium metabisulfite (SMB) and then washed thoroughly with 0.05M Tris buffer containing 0.05M SMB (Tris-SMB) pH 7.5.
3. Tissue sectioning: Vibratome sections will be cut through the region from the substantia to the raphe nuclei for the molecular detection of dopamine (DA) and tryptamine (T).
4. Application of anti-conjugated antiserum: Sections will be placed in Tris-SMB buffer, reduced using 0.1M sodium borohydride (in the same buffer), washed and then processed for immunocyto-chemistry using the peroxide / anti-peroxide (PAP) method. Following incubation with 3% non-specific serum in Tris-SMB for 1h at room temperature, the sections are incubated overnight at 4°C in Tris-SMB buffer containing 1% non specific serum, 0.02% Triton X100, diluted antibodies against DA and T (final dilutions 1/1,000 to 1/5,000). The T antiserum had previously been purified on the glutaraldehyde-conjugated protein carriers used during immunization and a monoclonal antibody to conjugated DA was produced.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

5. PAP procedure:
   Second antibody: After rinsing with Tris-NaCl buffer (0.05M Tris containing 0. 9% NaCl pH 7.4), sections will be incubated at 37°C in 1/100 dilutions of goat anti-mouse IgG antibodies and swine anti-rabbit IgG antibodies (DAKO) .
   PAP / Revelation: After another wash in Tris-NaCl buffer, sections will be incubated (1 hour at 37°C) with a 1/500 dilution of mouse PAP complex. The sections will be then washed again, and the reaction is developed in 0.05% 3-3'-diaminobenzidine (DAB) containing 0.01% hydrogene peroxide.
   After through washing in Tris-NaCl buffer, the sections will be incubated with a 1/1,000 dilution of rabbit PAP complex for 1 hour at 37°C, washed again and developed in a mixture of 0.025% DAB and 0.3% nickel ammonium sulphate containing 0.01% hydrogene peroxide. The sections will be mounted on slides using phosphate-buffered saline (PBS/glycerol, 1:3 v/v), placed under coverclips and observed under transmitted bright-field illumination.

   You must repeat the protocol; do step of PAP and develop with DAB after the second PAP with DADNi.

   Control sections will be prepared either by replacing one or the two the specific antibodies with non-immune rabbit or mouse serum, or by sequentially omitting each step of the immunological procedure. To determine the specificity of each of staining, the antibodies against DA and T will be incubated overnight at 4°C with each of the following conjugates: dopamine-glutaraldehyde-protein carrier (DA-G-Pc), HT-G-Pc, MT-G-Pc and T-G-Pc. Each mixture is then centrifuged at 10,000g for 15min, and the supernatants is used for the primary incubation in the immunocytochemical procedure described above. In these adsorption tests, the final dilutions of the antibodies against DA and T are identical to those used with the non-adsorbed antibodies. The final concentrations of each conjugated neurotransmitter (DA or HT, MT, T) is from 10-8 M to 10-9 M. Some sections were treated by the single peroxidase / antiperoxidase method. Thus, we compared both dopamine-T-Immunoreactivity (DA-IR) and T-IR in the same rat brain regions.

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Anti-Conjugated Adrenaline
RABBIT POLYCLONAL
AB-T069

Example of ELISA protocol used to test conjugated adrenaline:

1. Coating of conjugated adrenaline (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M , during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros), and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated adrenaline antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of ELISA protocol used to test conjugated octopamine:

1. Coating of conjugated octopamine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated octopamine antibodies will be diluted (1/2,000-1/10,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated Octopamine
RABBIT POLYCLONAL
AB-T070

Example of Immunohistochemistry used to test conjugated octopamine:

Detection of conjugated octopamine in dorsal unpaired median neurons innervating the colleterial glands of the female cockroach

1. Dissection: Cockroaches will be dissected under fixative: 1.5% glutaraldehyde in 0.1mol.l -1 cacodylate buffer containing 1% sodium metabisulfite (SMB), pH 7.2.
2. Post fixation: The abdominal nerve cord together with the gland will be removed and placed in the same fixative for 1-1. 5h at 20°C.
3. Application of anti-conjugated antiserum: The preparations will be preincubated with 10% normal swine serum in 0.05mol.l -1 Tris-HCl buffer containing 0.5% SMB and 0.5% TritonX-100 (TX) at pH 7.5 for 1h. Application of anti-conjugated Octopamine antiserum: The octopamine antiserum, diluted 1/1,000 in Tris-HCl-SMB-TX containing 1% swine serum, will be applied to whole mounts for 3 days at 20°C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

4. PAP procedure:
   Second antibody: After washing overnight in 0.05mol.l -1 Tris-HCl-TX, pH 7.5, the whole-mounts will be exposed to swine anti-rabbit IgG 1/80 for 12h.
   PAP: The tissue will be again washed overnight in Tris-HCl-TX, and a peroxidase/anti-peroxidase (PAP) complex will be applied at a concentration of 1/100 in Tris-HCl-TX with 1% swine serum for 12h.
   Revelation: After a final wash, in Tris-HCl buffer, pH7.5 (2x2h), the whole-mounts will be treated for 20min with 4-chloro-1-naphthol (4C1N) using 5mg of 4ICN dissolved in 1ml of methanol and 10ml of Tris-HCl pH7.5 containing 0.05% H2O2 (30%). The preparations will be washed in distilled water and mounted in neutral glycerine.

Example of ELISA protocol used to test conjugated tyramine:

1. Coating of conjugated tyramine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tyramine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values .

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Anti-Conjugated Tyramine
RABBIT POLYCLONAL
AB-T071

Example of Immunohistochemistry protocol used to test conjugated tyramine:

1. Under deep anesthesia, each rat will be perfused through the ascending aorta with 100ml of 0.01M phosphate-buffered saline (PBS) followed by 200ml of fixative containing 2% glutaraldehyde, 1% sodium metabisulfite, and 0.25% picric acid in 0.1M PB.
2. The brain will be removed and cut into several blocks, which will be postfixed in the same fixative for 8h, followed by a rinse for 3 days in PBS containing 20% sucrose and 1% sodium metabisulfite.
3. Sections will be cut with a cryostat in traverse plane.
4. The sections will be incubated in polyclonal antiserum against p-tyramine diluted (1/1,000-1/5,000) in PBS containing 0.3% Triton X-100 and sodium azide ar 4°C for 1 week; biotinylated rabbit IgG (Vector Laboratory, 1/1000) at 4°C for 12h; and avidin-biotin peroxidase complex (Vector Laboratory, 1/1000) at room temperature for 2h.
5. Peroxidase activity will be then revealed in 50mM Tris-HCl buffer (pH 7.6) containing 0.0003% H2O2, 0.01% 3,3'-diaminobenzidine-4HCl (DAB) and 1% nickel ammonium sulfate. The reaction will be terminated by washes in a Tris saline rinse.
6. Sections will be floated onto Tris solution on slides coated with 0.1% chromogelatin, dehydrated and coverslipped. Some serial sections will be stained by polyclonal anti-DA serum for the comparison of anti-p-tyramine staining.

Example of ELISA protocol used to test conjugated tyramine:

1. Coating of conjugated tyramine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tyramine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated 5-Methoxytryptamine
RABBIT POLYCLONAL
AB-T078

Example of ELISA procol used to test conjugated 5-methoxytryptamine:

1. Coating of conjugated 5-methoxytryptamine (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-methoxytryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated 5-methoxytryptamine:

Detection of conjugated 5-methoxytryptamine in rat brain

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated 5-methoxytryptamine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated 5-Methoxytryptophan
RABBIT POLYCLONAL
AB-T080

Example of ELISA protocol used to test conjugated 5-methoxytryptrytophan:

1. Coating of conjugated 5-methoxytryptrytophan (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-methoxytryptrytophan antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated 5-Methoxytryptophan
RABBIT POLYCLONAL
AB-T080

Example of Immunohistochemistry used to test conjugated 5-methoxytryptrytophan :

Perfusion Protocol for Adult Male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4ºC for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4º C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4ºC in the same mixed solution containing 5-methoxytryptophan antiserum (diluted as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated 4,5-Dihydroxytryptamine
RABBIT POLYCLONAL
AB-T081

Example of ELISA protocol used to test conjugated 4,5-dihydroxytryptamine:

1. Coating of conjugated 4,5-dihydroxytryptamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 4,5-dihydroxytryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated 4,5-Dihydroxytryptamine
RABBIT POLYCLONAL
AB-T081

Example of Immunohistochemistry used to test conjugated 4,5-dihydroxytryptamine:

Perfusion Protocol for Adult Male Sprague Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing 4,5-dihydroxytryptamine antiserum (diluted as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated 5-Hydroxytryptophol
RABBIT POLYCLONAL
AB-T082

Example of ELISA protocol used to test conjugated 5-hydroxytryptophol:

1. Coating of conjugated 5-hydroxytryptophol (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-hydroxytryptophol antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated 5-hydroxytryptophol:

Example of Protocol Perfusion for Adult male Sprague Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10-1 M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10-1 M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10-1 M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2).
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum.
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Phenyl-Acetic Acid
RABBIT POLYCLONAL
AB-T097

Example of ELISA protocol used to test conjugated phenyl acetic acid:

1. Coating of conjugated phenyl acetic acid (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated phenyl acetic acid antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated phenyl acetic acid:

Example of Protocol Perfusion for Adult male Sprague Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10-1 M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10-1 M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10-1 M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum.
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated 6-Hydroxytryptamine
RABBIT POLYCLONAL
AB-T103

Example of ELISA protocol used to test conjugated 6-hydroxytryptamine:

1. Coating of conjugated 6-hydroxytryptamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 6-hydroxytryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of Immunohistochemistry used to test conjugated 6-hydroxytryptamine:

Detection of conjugated 6-hydroxytryptamine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated 6-hydroxytryptamine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated 6-Hydroxydopamine
RABBIT POLYCLONAL
AB-T104

Example of ELISA protocol used to test conjugated 6-Hydroxydopamine:

1. Coating of conjugated 6-Hydroxydopamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05%Tween 20 (Acros) and SMB 0.001M during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated 6-hydroxydopamine antibodies will be diluted (1/2,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

Example of ELISA protocol used to test conjugated NO-L-Cystein:

1. Coating of conjugated NO-L-Cystein (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated NO-L-Cystein antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values (IC 50).

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Anti-Conjugated NO-L-Cysteine
MOUSE MONOCLONAL
AB-T125

Example of ELISA protocol used to test conjugated NO-L-Cystein:

1. Coating of conjugated NO-L-Cystein (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated NO-L-Cystein antibody will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-mouse (Sigma) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values (IC 50).

Example of ELISA protocol used to test conjugated L-arginine:

1. Coating of conjugated L-arginine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated L-arginine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values.

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Anti-Conjugated L-Arginine
RABBIT POLYCLONAL
AB-T126

Example of Immunohistochemistry used to test conjugated L-arginine:

Perfusion Protocol for Adult Male Sprague Dawley (weight around 0.5kg):

1. The animals can be deeply anesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing L-arginine antiserum (diluted as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Pseudomonas putida
RABBIT POLYCLONAL
AB-T138

Example of ELISA protocol used to test *Pseudomonas putida*:

1. Coating of Pseudomonas putida (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas putida serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas putida*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated Taurine
RABBIT POLYCLONAL
AB-T026

Example of Immunohistochemistry:

Detection of conjugated Taurine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%; pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Taurine antibodies: The final dilution is 1/2,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H2O2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

Example of ELISA protocol used to test conjugated D-Serine:

1. Coating of conjugated D-Serine (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed D-Serine antiserum will be diluted (1/10,000-1/25,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated D-Serine
RABBIT POLYCLONAL
AB-T048

Immunohistochemistry:

Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anaesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2 (two minutes).
   b) 600 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2 (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemistry Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing D-Serine antiserum (diluted 1/12,500; as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Proteus mirabilis
RABBIT POLYCLONAL
AB-T054

Example of ELISA protocol used to test *Proteus mirabilis*:

1. Coating of Proteus mirabilis (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Proteus mirabilis serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Proteus mirabilis*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Morganella morganii
RABBIT POLYCLONAL
AB-T055

Example of ELISA protocol used to test *Morganella morganii*:

1. Coating of Morganella morganii (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Morganella morganii serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Morganella morganii*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Pseudomonas aeruginosa
RABBIT POLYCLONAL
AB-T057

Example of ELISA protocol used to test *Pseudomonas aeruginosa*:

1. Coating of Pseudomonas aeruginosa (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas aeruginosa serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas aeruginosa*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Citrobacter koseri
RABBIT POLYCLONAL
AB-T056

Example of ELISA protocol used to test *Citrobacter koseri*:

1. Coating of Citrobacter koseri (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Citrobacter koseri serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Citrobacter koseri*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated D-Cysteine
RABBIT POLYCLONAL
AB-T050

Example of ELISA protocol used to test conjugated D-cysteine:

1. Coating of conjugated D-Cysteine (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed D-Cysteine antiserum will be diluted (1/5,000-1/15,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test conjugated D-Methionine:

1. Coating of conjugated D-Methionine (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed D-Methionine antiserum will be diluted (1/5,000-1/15,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Folic acid
RAT POLYCLONAL
AB-T147

Example of ELISA protocol used to test conjugated Folic acid:

1. Coating of conjugated folic acid (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed folic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Folic acid
RAT POLYCLONAL
AB-T147

Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
   c) 5,000ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
   d) 2,000ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing folic acid antiserum (diluted 1/500–1/1,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Thiamine
RAT POLYCLONAL
AB-T141

Example of ELISA protocol used to test conjugated Thiamine:

1. Coating of conjugated thiamine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed thiamine antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Thiamine
RAT POLYCLONAL
AB-T141

Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500 ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
   c) 5,000 ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
   d) 2,000 ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing thiamine antiserum (diluted 1/500–1/1,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min).
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Riboflavin
RAT POLYCLONAL
AB-T142

Example of ELISA protocol used to test conjugated Riboflavine:

1. Coating of conjugated riboflavin (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed riboflavin antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Riboflavin
RAT POLYCLONAL
AB-T142

Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500 ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
   c) 5,000 ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
   d) 2,000 ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing Riboflavin antiserum (diluted 1/500–1/1,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Phenylethylamine
RABBIT POLYCLONAL
AB-T073

Example of Immunohistochemistry:

Detection of conjugated Phenylethylamine in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated Phenylethylamine antibodies: The final dilution is 1/2,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

Example of ELISA protocol used to test *Pseudomonas marginalis*:

1. Coating of Pseudomonas marginalis (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas marginalis serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Pseudomonas marginalis
RAT POLYCLONAL
AB-T059

Example of Western blot protocol used to test *Pseudomonas marginalis*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of ELISA protocol used to test conjugated Myristic acid:

1. Coating of conjugated Myristic acid (50μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Wash with PBS (two times).
3. Preabsorbed Myristic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA (lipid free), 200μl by well plate (incubating during 2 hours at 37°C).
4. Wash with PBS (three times).
5. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2g/l of BSA (lipid free), will be applied by well plate (during one hour at 37°C).
6. Well plates will be rinsed with PBS containing 0.5% of Tween (three times).
7. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
8. The optical density will be measured at 492nm.

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Anti-Conjugated 5-Aminolevulinic Acid
RABBIT POLYCLONAL
AB-T087

Example of ELISA protocol used to test conjugated 5-Aminolevulinic acid:

1. Coating of conjugated 5-Aminolevulinic acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed 5-Aminolevulinic acid antiserum will be diluted (1/2,000-1/10,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test conjugated Pyridoxal (rd):

1. Coating of conjugated Pyridoxal (rd) (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pyridoxal (rd) antiserum will be diluted (1/2,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Pyridoxal
RABBIT POLYCLONAL
AB-T149

Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500ml of 1% paraformaldehyde in 0.1M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500ml of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4ºC (ten minutes).
   c) 5,000ml of cold 4% paraformaldehyde in 0.1M PB, pH 7.2 (fifty minutes).
   d) 2,000ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PB (0.1M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH3 (20%), H2O2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H2O2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing Pyridoxal antiserum (diluted 1/1,000–1/2,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rabbit immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H2O2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Pseudomonas aeruginosa
RAT POLYCLONAL
AB-T058

Example of ELISA protocol used to test *Pseudomonas aeruginosa*:

1. Coat maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing bacterial lysate of *Pseudomonas aeruginosa* (4µg/ml) for sixteen hours at 4°C. Lysates are formed through repeated freezing and sonification, followed by a protein concentration assay.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas aeruginosa serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas aeruginosa*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Pseudomonas blatchfordae
RAT POLYCLONAL
AB-T061

Example of ELISA protocol used to test *Pseudomonas blatchfordae*:

1. Coating of Pseudomonas blatchfordae (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas blatchfordae serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas blatchfordae*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Stenotrophomonas maltophilia
RAT POLYCLONAL
AB-T062

Example of ELISA protocol used to test *Stenotrophomonas maltophilia*:

1. Coating of Stenotrophomonas maltophilia (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Stenotrophomonas maltophilia serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Stenotrophomonas maltophilia*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H2O2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated D-Tyrosine
RABBIT POLYCLONAL
AB-T053

Example of ELISA protocol used to test conjugated D-Tyrosine:

1. Coating of conjugated D-Tyrosine (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed D-Tyrosine antiserum will be diluted (1/2,000-1/10,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Thioctic acid:

1. Coating of conjugated Thioctic acid (50µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Wash with PBS (two times).
3. Preabsorbed Thioctic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA (lipid free), 200µl by well plate (incubating during 2 hours at 37°C).
4. Wash with PBS (three times).
5. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2g/l of BSA (lipid free), will be applied by well plate (during one hour at 37°C).
6. Well plates will be rinsed with PBS containing 0.5% of Tween (three times).
7. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
8. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Rotenone:

1. Coating of conjugated Rotenone (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Rotenone serum will be diluted (1/2,000-1/5,000) in PBS Tween containing 1g/l BSA, and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Pyridoxine
RAT POLYCLONAL
AB-T144

ELISA protocol used to test conjugated Pyridoxine:

1. Coating of conjugated pyridoxine (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of phosphate buffer saline (PBS) (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed pyridoxine antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Pyridoxine
RAT POLYCLONAL
AB-T144

Example of Immunohistochemistry:

Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
   a) 500ml of 1% paraformaldehyde in 0.1M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
   b) 2,500ml of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C (ten minutes).
   c) 5,000ml of cold 4% paraformaldehyde in 0.1M PB, pH 7.2 (fifty minutes).
   d) 2,000ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
   e) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

   Around 50μm-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing Pyridoxine antiserum (diluted 1/1000–1/2,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min).
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Indole 3 Acetic Acid
RABBIT POLYCLONAL
AB-T129

ELISA protocol used to test conjugated Indole 3 acetic acid:

1. Coating of conjugated Indole 3 acetic acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Indole 3 acetic acid serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Gibberellic acid:

1. Coating of conjugated Gibberellic acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Gibberellic acid serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Abscisic acid:

1. Coating of conjugated Abscisic acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Abscisic acid serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Nicotinamide
RAT POLYCLONAL
AB-T150

ELISA protocol used to test conjugated Nicotinamide:

1. Coating of conjugated Nicotinamide (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of phosphate buffer saline (PBS) (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed nicotinamide antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of Tween.
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test conjugated 5-Methoxytryptamine:

1. Coating of conjugated 5-Methoxytryptamine (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed 5-Methoxytryptamine antiserum will be diluted (1/2,000-1/10,000)) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Agmatine:

1. Coating of conjugated Agmatine (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed conjugated Agmatine serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, 1g/l BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Homovanillic Acid
RAT POLYCLONAL
AB-T098

ELISA protocol used to test conjugated Homovanillic acid:

1. Coating of conjugated Homovanillic acid (50µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2 g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS (two times).
4. Preabsorbed conjugated Homovanillic acid serum will be diluted (1/5,000-1/10,000) in PBS containing 2g/l BSA, 5% glycerol and 0.2g/l f of conjugated N.Acetyl-Cystéine (gemacbio), 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS -Tween containing 5g/l BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS-Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Formiate:

1. Coating of conjugated Formiate (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS (three times).
4. Preabsorbed conjugated Formiate serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 2,5g/l BSA, 1g/l BSA-G and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated Phenyl-acetic acid:

1. Coating of conjugated Phenyl-acetic acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS-Tween (pH 7.3) containing 10% glycerol and 1g/l of BSA (Acros) (1h at 37°C).
3. Wash with PBS-Tween (three times).
4. Preabsorbed conjugated Phenyl-acetic acid serum will be diluted (1/5,000-1/10,000) in PBS-Tween containing 1g/l BSA and 10% glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS-Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS-Tween containing 5g/l BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS-Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Gamma-Amino-Butyric Acid (GABA)
RAT POLYCLONAL
AB-T158

ELISA protocol used to test conjugated GABA:

1. Coating of conjugated GABA (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with a solution of PBS-Tween (pH 7.3) containing 10% glycerol and 1 g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS-Tween (three times).
4. Preabsorbed conjugated GABA serum will be diluted (1/5,000-1/10,000) in PBS-Tween containing 1g/l BSA, 1g/l BSA-reduced glutaraldehyde and 10% glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS-Tween containing 1g/l BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS-Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

ELISA protocol used to test conjugated D-Aspartic acid:

1. Coating of conjugated D.Aspartic acid (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with a solution of PBS-Tween (pH 7.3) containing 10% glycerol and 1g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS-Tween (three times).
4. Preabsorbed conjugated D-Aspartic acid serum will be diluted (1/5,000-1/10,000) in PBS-Tween containing 1g/l BSA, 1g/l BSA-reduced glutaraldehyde and 10% glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS-Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS-Tween containing 1g/l BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS-Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Valeric Acid
RABBIT POLYCLONAL
AB-T089

ELISA protocol used to test conjugated Valeric acid:

1. Coating of conjugated Valeric acid (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed valeric acid serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test conjugated ascorbic acid:

1. Coating of conjugated ascorbic acid (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (three times).
4. Preabsorbed ascorbic acid antiserum will be diluted (1/2,000-1/5,000) in PBS tween containing 1g/l BSA and 5% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 1g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween.
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values (IC 50).

Example of ELISA protocol used to test conjugated succinic acid:

1. Coating of conjugated succinic acid (15µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (two times).
4. Preabsorbed succinic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values (IC 50).

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Anti-Conjugated 1,3 Butadiene
RABBIT POLYCLONAL
AB-T102

ELISA protocol used to test conjugated 1,3-Butadiene:

1. Coating of conjugated 1,3-Butadiene (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed 1,3-Butadiene antiserum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test conjugated NO_2-tyrosine-DP:

Instructions for use: dark conditions

1. Coating of conjugated NO_2-tyrosine-DP (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated NO_2-tyrosine-DP antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated NO_2-Tyrosine-Diaminopentane
RAT POLYCLONAL
AB-T112

Example of Immunochemistry protocol used to test conjugated NO_2-tyrosine-DP:
Instructions for use: dark conditions

Example of Perfusion protocol for Adult male Sprague Dawley (weight 0.5 kg):
1. The animals can be deeply anaesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1}M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10^{-1}M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1}M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:
1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum (1/1,000-1/5,000).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6) (10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Example of ELISA protocol used to test conjugated Taurine-GA:
1. Coating of conjugated Taurine-GA (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated Taurine-GA antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Taurine
RAT POLYCLONAL
AB-T028

Example of Immunochemistry protocol used to test conjugated Taurine-GA:

Example of Perfusion protocol for Adult male Sprague Dawley (weight 0.5 kg):

1. The animals can be deeply anaesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1}M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10^{-1}M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1}M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum (1/1,000-1/5,000) .
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min).
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Example of ELISA protocol used to test conjugated Taurine:

1. Coating of conjugated taurine-GA-DP (15μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated taurine-GA-DP antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Taurine-GA-Diaminopentane
RABBIT POLYCLONAL
AB-T027

Example of Immunochemistry protocol used to test conjugated Taurine:

Example of Perfusion protocol for Adult male Sprague Dawley (weight 0.5 kg):

1. The animals can be deeply anaesthetized (for example with urethane 1-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1}M, pH 5.4, and with the following solutions:
   a) 200ml of a solution containing MES 10^{-1}M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1}M (two minutes).
   b) 800-1000ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000ml of cold 4% paraformaldehyde (Merck) in 0.1M PB, pH 7.2, (ten minutes).
   d) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum (1/1,000-1/5,000) .
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector; Serotec) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3'-diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Example of ELISA protocol used to test conjugated Kainic acid:

1. Coating of conjugated Kainic acid (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Kainic acid antiserum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Valproic Acid
RABBIT POLYCLONAL
AB-T160

ELISA protocol used to test conjugated Valproic acid:

1. Coating of conjugated Valproic acid (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS (three times).
4. Preabsorbed conjugated Valproic acid serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of ELISA protocol used to test Hafnia alvei:

1. Coating of Hafnia alvei (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Hafnia alvei serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Hafnia alvei
RABBIT POLYCLONAL
AB-T156

Example of Western blot protocol used to test Hafnia alvei:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C.
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C.
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C.
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C.
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at 37°C.
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark.
10. Stop the reaction by addition of distilled water.

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of ELISA protocol used to test Enterobacter cloacae:

1. Coating of Enterobacter cloacae (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Enterobacter cloacae serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Enterobacter cloacae
RABBIT POLYCLONAL
AB-T157

Example of Western blot protocol used to test Enterobacter cloacae:

Membrane Blocking, Antiboby Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of Immunochemistry:

Example of Perfusion protocol for Adult male Sprague Dawley (weight 0.5 kg):

1. The animals can be deeply anesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1} M, pH 5.4,and with the following solutions:
   a) 200 ml of a solution containing MES 10^{-1} M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1} M (two minutes).
   b) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
   d) Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of Immunohistochemical Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing the diluted antiserum (AB-T044 – 1/2000 to 1/5000).
5. Then, the sections will be washed in PBS (30 min).
6. After that incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated D-Alanine
RABBIT POLYCLONAL
AB-T049

Example of ELISA protocol used to test conjugated D-Alanine:

1. Coating of conjugated D-Alanine (10μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed D-Alanine antiserum will be diluted (1/5,000-1/15,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated D-Alanine
RABBIT POLYCLONAL
AB-T049

Example of Immunochemistry:

Example of Perfusion protocol for Adult male Sprague Dawley (weight 0.5 kg):

1. The animals can be deeply anaesthetized for example with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (two minutes).
   b) 600 ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of Immunohistochemistry Protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing D-Alanine antiserum (diluted 1/7,500; as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Pseudomonas aureofasciens
RAT POLYCLONAL
AB-T060

Example of ELISA protocol used to test *Pseudomonas aureofasciens*:

1. Coating of *Pseudomonas aureofasciens* (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed *Pseudomonas aureofasciens* serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas aureofasciens*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37° C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Staphylococcus aureus
RABBIT POLYCLONAL
AB-T161

Example of ELISA protocol:

1. Coating of Staphylococcus aureus antigens (4 µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05 M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 5 g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Staphylococcus aureus antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5 g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1 M/phosphate 0.2 M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50 µl of 2 M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas aureofasciens*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti- Staphylococcus aureus antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1 µg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated Ibuprofen
RABBIT POLYCLONAL
AB-T162

Example of ELISA protocol:

1. Coating of Ibuprofen-BSA conjugate (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 2.5g/l BSA (Acros) and 0.05% Tween 20 (across) (one hour at 37°C).
3. Wash with PBS containing 0.05% of Tween (PBS Tween) (three times).
4. Anti-conjugated Ibuprofen antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol (Membrane blocking, antibodies incubations and detection):

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti-conjugated Ibuprofen antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated Ibuprofen
RABBIT POLYCLONAL
AB-T162

Example of immunohistochemistry protocol:

Example of Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anaesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1} M, pH 5.4, and with the following solutions:
   a) 200 ml of a solution containing MES 10^{-1} M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1} M (two minutes).
   b) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
   d) Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of immunohistochemical protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing anti-conjugated Ibuprofen antibodies (diluted 1/1,000 to 1/5,000; as recommended dilution).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rabbit immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Example of ELISA protocol:

1. Coating of conjugated Phytanic acid (20μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS (three times).
4. Anti-conjugated Phytanic acid antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 1g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Conjugated Phytanic Acid
RABBIT POLYCLONAL
AB-T163

Example of immunohistochemistry protocol:

Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anaesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka) 10^{-1} M, pH 5.4, and with the following solutions:
   a) 200 ml of a solution containing MES 10^{-1} M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros] 10^{-1} M (two minutes).
   b) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
   c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
   d) Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Example of immunohistochemical protocol:

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing the diluted anti-conjugated Phytanic acid antibodies (1/1,000 – 1/5,000).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rabbit immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

Example of ELISA protocol used to test *Escherichia coli* O26:B6:H11:

1. Coating of *Escherichia coli* O26:B6:H11 (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 5g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed *Escherichia coli* O26:B6:H11 serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/5,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Escherichia coli O26:B6:H11
RABBIT POLYCLONAL
AB-T164

Example of Western blot protocol used to test *Escherichia coli* O26:B6:H11:

Membrane Blocking, Antiboby Incubations and Detection of Proteins:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with the antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of ELISA protocol used to test *Escherichia coli* O26:B6:

1. Coating of *Escherichia coli* O26:B6 (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 5g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed *Escherichia coli* O26:B6 serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/5,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Escherichia coli O26:B6
RABBIT POLYCLONAL
AB-T165

Example of Western blot protocol used to test *Escherichia coli* O26:B6:

Membrane Blocking, Antiboby Incubations and Detection of Proteins:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with the antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of ELISA protocol used to test *Alkalescens dispar*:

1. Coating of *Alkalescens dispar* antigens (4μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 5g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed *Alkalescens dispar* anti-serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5g/l BSA, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/5,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200μl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50μl of 2M HCl.
9. The optical density will be measured at 492nm.

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Anti-Alkalescens dispar
RABBIT POLYCLONAL
AB-T166

Example of Western blot protocol used to test *Alkalescens dispar*:

Membrane Blocking, Antiboby Incubations and Detection of Proteins:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with the antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of Immunohistochemistry applications:

Detection of L-Norephedrine in rat brain:

1. Perfusion: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:
   solution A (30ml): 200-300ml/min
   solution B (500ml): 200-300ml/min
   Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2
   Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. Tissue sectioning: Cryostat or vibratome sections can be used.
4. Reduction step: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. Application of anti-conjugated L.Norephedrine antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.

6. PAP procedure:
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   PAP: Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

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Anti-Conjugated L-Norephedrine
RABBIT POLYCLONAL
AB-T167

Example of Western blot protocol:

Membrane blocking, antibodies incubations and detection:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti-conjugated L.Norephedrine antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1μg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50μl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

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Anti-Conjugated Uric Acid
RABBIT POLYCLONAL
AB-T168

Example of Immunochemistry protocol:

Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anaesthetized for example with urethane (0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 100 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution:
   a) 300 ml of cold 4% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (two minutes).
   b) 600 ml of cold 4% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, (ten minutes).
   c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.
   d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

   Around 50 μm-thick serial sections will be obtained, kept at 4°C in PBS (0.1 M, pH 7.2) and processed for immunostaining.

Example of immunohistochemical protocol

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing the diluted anti-conjugated Uric acid antibodies (1/1,000-1/5,000).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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Anti-Conjugated Uric Acid
RABBIT POLYCLONAL
AB-T168

Example of Western blot protocol:

Membrane blocking, antibodies incubations and detection:

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti-conjugated Uric acid antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1µg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20

Example of ELISA protocol:

1. Coating of Uric acid(BSA) conjugate (10µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 2.5g/l BSA (Acros) and 0.05% Tween 20 (across) (one hour at 37°C).
3. Wash with PBS containing 0.05% of Tween (PBS Tween) (three times).
4. Anti-conjugated Uric acid antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1M/phosphate 0.2M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50µl of 2M HCl.
9. The optical density will be measured at 492nm

A ADVANCED TARGETING SYSTEMS

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Anti-Anti-Conjugated L-Glutamate
RABBIT POLYCLONAL
AB-T021

Example of Immunochemistry protocol:

Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):

1. The animals can be deeply anaesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Perfused via the ascending aorta with 50 ml of NaCl 9g/l (Heparinized) andpass through the system 800 -1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
3. Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

Immunohistochemistry

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing the diluted anti-anti conjugated L.Glutamate antibodies(1/1,000-1/5,000).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunogammaglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H_2O_2 using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).