Advanced Targeting Systems

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 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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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.