Advanced Targeting Systems

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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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.