



**Anti-Conjugated L-Glutamate
MOUSE MONOCLONAL
AB-T12**

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

1. Coating of conjugated L-glutamate (15 μ g/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, 200 μ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 μ l 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 200 μ l 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 50 μ l 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 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 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 (NaBH₄, 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-aurine 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.