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Anti-Conjugated Acetylcholine MOUSE MONOCLONAL

Catalog Number: AB-T029

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

1. Coating of conjugated choline-glutaric acid (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 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, 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-labelled 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 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 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 metabisulfite 10g/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.