ADVANCED TARGETING SYSTEMS

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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.