ADVANCED TARGETING SYSTEMS

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Anti-Conjugated GABA (Gamma-Aminobutyric acid)
RABBIT POLYCLONAL
AB-T10

Example of ELISA protocol used to test conjugated GABA:

1. Coating of conjugated GABA (10μg/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 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Anti-conjugated GABA antibodies will be diluted (1/1,000-1/5,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G 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-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with 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.

Example of Immunohistochemistry used to test conjugated GABA:

Detection of conjugated GABA in rat brain

1. Perfusion: The rat is anaesthetized 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. Application of anti-conjugated GABA antibodies: The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, 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 H_2O_2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.