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**Anti-Conjugated Phenylethylamine
RABBIT POLYCLONAL
AB-T073**

Example of Immunohistochemistry:

Detection of conjugated Phenylethylamine 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. 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 Phenylethylamine antibodies: The final dilution is 1/2,000 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.

6. 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 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.