

**Anti-Conjugated GABA (Gamma-Aminobutyric acid)  
RABBIT POLYCLONAL**

**Catalog Number:** AB-T10

**Example of ELISA protocol used to test conjugated GABA:**

1. Coating of conjugated GABA (10 $\mu$ 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 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, 200 $\mu$ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 $\mu$ l 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 200 $\mu$ 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 $\mu$ l 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<sub>2</sub>O<sub>2</sub> is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.