

**Anti-Conjugated Glycine
RABBIT POLYCLONAL
AB-T023**

Example of ELISA protocol used to test conjugated glycine:

1. Coating of conjugated glycine ($10\mu\text{g/ml}$) 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 glycine 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, $200\mu\text{l}$ by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. $200\mu\text{l}$ 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 $200\mu\text{l}$ 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 $50\mu\text{l}$ of 2M HCl.
9. The optical density will be measured at 492nm .

Example of Immunohistochemistry used to test conjugated glycine:

Detection of conjugated Glycine 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 Glycine 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.

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 ($25\text{mg}/100\text{ml}$) (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 transferring sections in 5ml of Tris 0.05M .