ANTI-CONJUGATED GABA (Gamma-Aminobutyric acid) ANTIBODIES

CATALOG NUMBER : AP027

LOT NUMBER :

TARGET : Conjugate GABA

IMMUNOGEN : Synthetic GABA conjugated to protein carrier (Pc)

SPECIFICITY : Using a conjugate GABA-(Pc), antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity ratio (a)</th>
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<tbody>
<tr>
<td>GABA-G-(Pc)</td>
<td>1</td>
</tr>
<tr>
<td>β-Alanine-G-(Pc)</td>
<td>1/10,000</td>
</tr>
<tr>
<td>Glycine-G-(Pc)</td>
<td>1/20,000</td>
</tr>
<tr>
<td>D-Aspartate-G-(Pc)</td>
<td>1/&gt; 50,000</td>
</tr>
<tr>
<td>D-Glutamate-G-(Pc)</td>
<td>1/&gt; 10,000</td>
</tr>
</tbody>
</table>

(a) : GABA-G-(Pc) concentration/unconjugated or conjugated aminoacid concentration at half displacement ; G = Glutaraldehyde

RAISED IN : Rabbit

CLONALITY : Polyclonal

ISOTYPE : IgG

PURITY : Antiserum previously preabsobed on protein carriers, and purified.

FORM : Lyophilized

TESTED APPLICATIONS : Elisa, Immunocytochemistry

APPLICATIONS NOTES :
Recommended dilutions for Elisa (1/1,000-1/5,000)
Recommended dilutions for Immunocytochemistry (1/1,000-5,000)
Recommended dilutions for Western Blot (1/1,000-1/2,000)

RESEARCH AREAS : Neurobiology, Pharmacology, Biochemistry, Neurological diseases

STORAGE INSTRUCTIONS :
Lyophilized antibodies are stable at least 2 years.
After reconstitution with 50µl of distilled water and 50µl of glycerol, the aliquot can be repeated freezeed (up to five times).

CORRESPONDING ANTIGEN :
Gemac sells the corresponding antigen : GABA (Gamma aminobutyric acid) conjugate (code number: AG027).
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EXAMPLES OF MATERIALS AND METHODS

**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 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-labelled 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 Immunocytochemistry applications 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 sectionning** : 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.

   N.B. : 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 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.

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Gemacbio sells the L and D-Aspartate, Taurine, L and D-Glutamate antibodies raised in rabbit / rat: used together, these tools could be helpful for immunocytochemistry double labelling.

- **Western blot**: Antibodies can be used for western blot