PRODUCT INFORMATION

ANTI-CONJUGATED 5-HYDROXYTRYPTAMINE (SEROTONIN) ANTIBODIES

Ref: AP014

TARGET: Conjugated 5-Hydroxytryptamine

IMMUNOGEN: Synthetic 5-Hydroxytryptamine conjugated to protein carrier (PC)

SPECIFICITY: Using a conjugate 5-Hydroxytryptamine-PC, antibody specificity was performed with an ELISA by competition experiments with the following compounds:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cross-reactivity</th>
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</thead>
<tbody>
<tr>
<td>5-Hydroxytryptamine-PC</td>
<td>1</td>
</tr>
<tr>
<td>5-Methoxytryptamine-PC</td>
<td>1/&gt;25</td>
</tr>
<tr>
<td>Tryptamine-PC</td>
<td>1/&gt;50</td>
</tr>
<tr>
<td>5-Hydroxytryptophan-PC</td>
<td>1/&gt;175</td>
</tr>
<tr>
<td>5-Hydroxytryptamine (free)</td>
<td>1/&gt;1,150</td>
</tr>
<tr>
<td>L-Tryptophan-PC</td>
<td>1/&gt;3,500</td>
</tr>
<tr>
<td>5-Methoxytryptophan-PC</td>
<td>1/&gt;20,000</td>
</tr>
</tbody>
</table>

(a): 5-Hydroxytryptamine-PC concentration /other conjugated indolealkylamines concentration at half displacement
RAISED IN: Rabbit

CLONALITY: Polyclonal

ISOTYPE: IgG

PURITY: Antiserum preabsorbed on protein carriers, purified by ammonium sulfate and/or by high trap column

FORM: Lyophilized

STORAGE INSTRUCTIONS:
Lyophilized vial must be stored at 4°C in a dry area. After reconstitution with 50% of distilled water and 50% of glycerol, the aliquot can be stored at -20°C, and is stable at least 2 years.

RESEARCH AREAS: Neurobiology, Biochemistry, Pharmacology, Neurodegenerative diseases

TESTED APPLICATION: Immunocytochemistry, Immunohistochemistry. Optimal dilutions should be determined by each laboratory for each application.

CORRESPONDING ANTIGEN:
Gemac sell the corresponding antigen: 5-Hydroxytryptamine conjugate (ref: AG014)

CORRESPONDING ANTI-IDIOPTYPIC ANTIBODY:
Gemac sell the anti-idiotypic antibody: Anti-Anti-Serotonin (Mouse) (ref: AIM010)
REFERENCES


BENNIS M. GAMRANI H., GEFFARD M., CALAS A. and KAH O. The distribution of 5-HT immunoreactive systems in the brain of a saurian, the chameleon. J. Hirnforsch, (1990), 31, 563-574.


RAJAOFETRA N., PASSAGIA J.G., MARLIER L., POULAT P., PELLAS F., SANDILLON F., VERSCHUERE B., GOUY D., GEFFARD M. and PRIVAT A. Serotoninergic, noradrenergic and


EXAMPLE OF IMMUNOCYTOCHEMISTRY APPLICATION

Detection of conjugated 5-Hydroxytryptamine (serotonin) in rat brain

1- Perfusion: The rat is anesthetized 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 5-Hydroxytryptamine 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.

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 1/1000 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.