PRODUCT INFORMATION

ANTI-CONJUGATED HISTAMINE ANTIBODIES

Ref: AP032

TARGET: Conjugated Histamine

IMMUNOGEN: Synthetic Histamine conjugated to protein carrier (PC)

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cross-reactivity ratio (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine-PC</td>
<td>1</td>
</tr>
<tr>
<td>1-Methylhistamine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>3-Methylhistamine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>L-Histidine-PC</td>
<td>1/&gt;50,000</td>
</tr>
</tbody>
</table>

(a): Histamine-PC concentration/other conjugated aminoacid 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 50μL of distilled water and 50μL of glycerol, the aliquot can be stored at -20°C, and is stable at least 2 years.

RESEARCH AREAS: Neuroscience

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

CORRESPONDING ANTIGEN: Gemac sell the corresponding antigen: Histamine conjugate (ref: AG032)

REFERENCES


EXAMPLE OF IMMUNOCYTOCHEMISTRY APPLICATION

Detection of conjugated Histamine 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 Histamine 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 H$_2$O$_2$ is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5mL of Tris 0.05M.