ANTI-CONJUGATED HISTAMINE ANTIBODY

CODE NUMBER : MBS358003

LOT NUMBER : 03050901

DESCRIPTION : Monoclonal antibody was obtained after BALB/c mouse immunisation with the conjugate : Histamine-Protein Carrier and hybridization of spleen cells with the myeloma cell line SP2/O/Ag14. Ascite production was performed in BALB/c mice.

TARGET : Conjugated Histamine

IMMUNOGEN : Synthetic Histamine conjugated to protein carrier (PC)

SPECIFICITY : Using a conjugate Histamine-PC, antibody specificity was performed with an ELISA test 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-Methyl-Histamine-PC</td>
<td>1</td>
</tr>
<tr>
<td>Histidine-PC</td>
<td>1/&gt;50,000</td>
</tr>
</tbody>
</table>

(a) : Histamine-PC concentration/ other conjugated amino acids concentration at half displacement

IC50 = 1 X 10^-9 M

RAISED IN : Mouse

CLONALITY : Monoclonal

ISOTYPE : IgG, Kappa

PURITY : The ascitic fluid was purified by ammonium sulfate precipitation

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 : Neurobiology, Immunology

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

CORRESPONDING ANTIGEN :
The corresponding antigen: Histamine conjugate (code number: MBS358324)

REFERENCE

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EXAMPLES OF MATERIAL AND METHODS

- **Example of immunohistochemistry protocol**

  **Perfusion** (Example for Adult male Sprague Dawley (weight around 0.5 kg)).
  1. The animals can be deeply anaesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
  2. Perfused via the ascending aorta with 50 ml of NaCl 9g/l (Heparinized) and pass through the system 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
  3. Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

  **Immunohistochemistry**
  1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH\(_3\) (20%), H\(_2\)O\(_2\) (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H\(_2\)O\(_2\) and 66% of methanol).
  2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).
  3. Pre-incubate for 30 min in PBS containing 2-10% (variable to adjust) of normal horse serum and 0.3% of Triton X-100 (mixed solution).
  4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing the diluted (1/500-1/1000) anti-conjugated Histamine antibody.
  5. Then, the sections will be wash in PBS (30 min).
  6. After that we will incubate for 60 min at room temperature with biotinylated anti-(species) immunoglobulin (Vector) diluted 1/200 in PBS.
  7. Wash during 30 min with PBS.
  8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain) in the mixed solution.
  9. After that we will wash the sections in PBS (30 min).
  10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
  11. The tissue-bound peroxidase will be developed with H\(_2\)O\(_2\) using 3, 3’ diaminobenzidine as chromogen.
  12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).

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