PRODUCT INFORMATION

ANTI-CONJUGATED L-DIHYDROXYPHENYLALANINE (L-DOPA) ANTIBODY

Ref: AM004

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

TARGET: Conjugated L-Dihydroxyphenylalanine (L-DOPA)

IMMUNOGEN: Synthetic L-DOPA conjugated to protein carrier (PC)

SPECIFICITY: Using a conjugate L-DOPA-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>
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</thead>
<tbody>
<tr>
<td>L-DOPA-PC</td>
<td>1</td>
</tr>
<tr>
<td>Dopamine-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>Noradrenaline-PC</td>
<td>1/&gt;50,000</td>
</tr>
<tr>
<td>Tyrosine-PC</td>
<td>1/&gt;50,000</td>
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(a): L-DOPA-PC concentration/other conjugated catecholamine concentration at half-displacement
RAISED IN: Mouse

CLONALITY: Monoclonal

ISOTYPE: IgG1, Kappa

PURITY: The ascitic fluid was purified by ammonium sulfate precipitation 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: Neurobiology, Pharmacology, Thrombosis research

TESTED APPLICATIONS: Immunocytochemistry

CORRESPONDING ANTIGEN: Gemac sell the corresponding antigen: Anti-L-DOPA (ref: AG004)

CORRESPONDING ANTI-IDIOPTYPIC ANTIBODIES:
Gemac sell the anti-idiotypic antibodies: Anti-Anti-L-DOPA (Rabbit) (ref: AIP022)

REFERENCE
Examples of cytochemical protocol

Detection of conjugated L-DOPA in rat brain

1- **Perfusion**: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 100ml 0.1M cacodylate buffer containing 5% glutaraldehyde (G) and 0.9% sodium metabisulfite (SMB) pH 7.5.

2- **Post fixation**: Brains will be removed quickly and post-fixed for 2 hours at 4°C in the same solution.

3- **Tissue sectioning**: Serial transverse vibratome sections will be cut in Tris-SMB.

4- **Application of anti-conjugated antiserum**: Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB pH 7.5).

Then, the sections will be washed with in the same solution and incubated in Tris-SMB containing 3% non-specific serum (1h at 4°C):

Application of anti-conjugated L-DOPA antibodies: The sections will be incubated floating free in plastic wells containing rabbit conjugated L-DOPA antiserum (1/1000 to 1/5,000) or monoclonal anti conjugated DA antibody (1/1000 to 1/5,000) for 2 days at 4°C in Tris-SMB buffer pH 7.4.

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: After rinsing 3 times for 10min in 0.1M Tris buffer containing 0.9% NaCl (Tris-NaCl) pH 7.4, the sections will be incubated with swine anti-rabbit IgG antibodies (Dako) or goat anti-mouse IgG antibodies (Dako). Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum .

PAP: Rinsed again, sections will be then incubated (1 hour at 37°C) in Tris-NaCl with a 1/500 dilution of rabbit or mouse peroxidase anti-peroxidase (PAP) complex (Dako).

Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3’ diaminobenzidine (DAB ,Sigma) and 0.01% hydrogen peroxide (Merck).

The reaction will be stopped by the transfer of the sections in Tris-NaCl buffer.

Gemac sell the same antibodies raised in rabbit: used together, these tools could be helpful for double labelling in immunocytochemistry.

*Double detection of conjugated L-DOPA and Dopamine (DA) in rat brain*

1- **Perfusion**: The rat will be deeply anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the 500ml of 5% glutaraldehyde (G), 0.9% sodium metabisulfite (SMB) solution in 0.1 cacodylate buffer pH 7.4.

2- **Post fixation**: 2H, 4°C in the same fixative solution.
3- **Tissue sectioning:** Cryostat or vibratome sections can be used.

4- **Application of anti-conjugated antiserum:** Sections will be reduced in 0.05M Tris buffer containing 0.9% SMB (Tris-SMB).

Then, the sections will be washed in the same solution (12h, 4°C) and incubated in Tris-SMB containing 3% non specific serum and 0.1% Triton X100 (8h at 4°C).

Application of anti-conjugated L-DOPA antibodies: Free Floating adjacent sections will be incubated (24h, 4°C) with a monoclonal antibody against G-conjugated L-DOPA (1/1,000 to 1/5,000), with a monoclonal antibody against G-conjugated DA (1/1,000 to 1/5,000), and with both. Antibodies will be diluted in Tris-SMB, 1% non-specific serum, 0.2% Triton X100 solution.

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: After rinsing, sections will be incubated (12 hours at 4°C) with respectively swine anti-rabbit IgG antibodies (Dako), goat antimouse IgG antibodies (Dako) and both. Secondary antibodies will be diluted (1/500) in Tris buffer, 0.9% NaCl, pH 7.4 containing 1% non-specific serum.

PAP: Rinsed again, sections will be then incubated (1 hour at 37°C) with a 1/1,000 dilution of rabbit peroxidase anti-peroxidase (PAP) complex (Dako) for single L-DOPA detection and 1/500 dilution of PAP mouse complex for DA detection.

Revelation: After a final rinse, coloration will be revealed in a Tris-NaCl solution (pH 7.6) containing 0.05% 3-3' diaminobenzidine (DAB, Sigma) plus cobalt chloride (Sigma, 10mg/20ml) and 0.01% hydrogen peroxide (30vol., Merck).

You must repeat the protocol: it needs that you must do step of PAP and develop with DAB after the second PAP with DADNi.

For the double detection of L-DOPA and DA, the sections which have received anti-DA together with L-DOPA antibodies and then anti-rabbit together with anti-mouse secondary antibodies, will be incubated with PAP mouse complex, then revealed in DAB plus cobalt chloride, giving a dark-blue color. These staining sections will be then washed thoroughly (12 hours at 4°C) and incubated with PAP rabbit complex, then revealed in a 0.1% DAB solution giving a yellow-brown coloration.