PRODUCT INFORMATION

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

Ref: AP004

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>
</tr>
</thead>
<tbody>
<tr>
<td>L-DOPA-PC</td>
<td>1</td>
</tr>
<tr>
<td>α-methyl-L-DOPA-PC</td>
<td>1/&gt;2,200</td>
</tr>
<tr>
<td>3-O-methyl-L-DOPA-PC</td>
<td>1/&gt;50,000</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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</tbody>
</table>

(a): L-DOPA-PC concentration/other conjugated catecholamine 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: Neurobiology, Thrombosis research

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

CORRESPONDING ANTIGEN:
Gemac sell the corresponding antigen: L-Dihydroxyphenylalanine conjugate (ref: AG004)

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


EXAMPLE OF CYTOCHEMISTRY APPLICATION

Detection of conjugated L-DOPA 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- 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 L-DOPA 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 1/1,000 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.

Gemac sell the same and other antibodies to conjugated small molecules raised in mouse: used together, these tools could be helpful for immunocytochemistry double labelling.
Double detection of conjugated L-DOPA and Dopamine 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).

5- **Application of anti-conjugated L-DOPA antibodies:** Free floating adjacent sections will be incubated (24h, 4°C) with a polyclonal antiserum against conjugated L-DOPA (1/1,000 to 1/5,000), with a monoclonal antibody against conjugated DA (1/1,000 to 1/5,000), and with both. Antisera 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.

6- **PAP procedure:**

Second antibody: After rinsing, sections will be incubated (12 hours at 4°C) with respectively swine anti-rabbit IgG antibodies (DAKO), goat anti-mouse 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% hydrogene 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.