POLYCLONAL ANTI-CONJUGATED DOPAMINE ANTIBODIES (rabbit)

Data Sheet

Code number: AP001

Polyclonal antisera were raised in rabbits after immunisation with the conjugates: Dopamine-Gluteraldehyde-Carriers.

Specificity
Using a conjugate Dopamine-Gluteraldehyde-Protein, antibody specificity was performed with an ELISA test by competition experiments with the following compounds:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity ratio (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine-G-BSA</td>
<td>1</td>
</tr>
<tr>
<td>Noradrenaline-G-BSA</td>
<td>1/159</td>
</tr>
<tr>
<td>L-DOPA-G-BSA</td>
<td>1/400</td>
</tr>
<tr>
<td>Octopamine-G-BSA</td>
<td>1/400</td>
</tr>
<tr>
<td>Tyramine-G-BSA</td>
<td>1/5,000</td>
</tr>
<tr>
<td>Adrenaline-G-BSA</td>
<td>1/5,000</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1/5,000</td>
</tr>
</tbody>
</table>

(a): Dopamine-G-BSA concentration/unconjugated or conjugated catecholamine concentration at half displacement.
G = Glutaraldehyde, BSA = Bovine Serum Albumin.

Recommended dilution
The antiserum was tested using the free floating PAP technique on rat dopaminergic areas. The anti-conjugated Dopamine antibodies gave a good staining between a 1/2,000-1/5,000 dilution in these areas.

Applications
Immunohistochemistry, immunocytochemistry.

Storage and handling
Antisera were aliquoted (100µl) and stored at -20°C or lower. They are stable at least 2 years. Each aliquot can be defreezed and refreezed up to 5 times. It can be prediluted 10X in PBS containing 0.1% merthiolate or a mixture PBS/glycerol (vol/vol). These solutions were stable at +4°C for 2 months. Lyophilized antisera are stable at least 1 year. Antisera could be reconstituted with 100µl pure water when the solution is completely used. For a storage at +4°C, use pure water with 0.1% merthiolate. This solution is stable at +4°C for 2 months. For a storage at -20°C, a mixture of water/glycerol (vol./vol.) is preferred.

Corresponding antigen
Gemac sells the corresponding antigen:
Dopamine conjugate (code number: AG001)
Immunohisto and cytochemical applications

Detection of conjugated Dopamine in rat brain

**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

**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).

**Tissue sectionning**: Cryostat or vibratome sections can be used.

**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.

Application of anti-conjugated Dopamine antibodies: The final dilution is 1/2,000 to 1/5,000 in solution C containing triton X100 0.1%, 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.

**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.

**References**

• KAH O., DUBBOURG P., ONTENIENTE B., GEFFARD M. and CALAS A. The dopaminergic innervation of the goldfish pituitary. An immunocytochemical study at the electron-microscope level using antibodies against dopamine, Cell & Tissue Research, (1986) 244, 577-582.


• ARAI R., KOJIMA Y., GEFFARD M., KITAHAMA K. and MEADA T. Combined use of silver staining of the retrograde tracer WGApoHRP-Au and pre-embedding immunocytochemistry for electron microscopy: demonstration of dopaminergic terminals in synaptic contact with striatal neurons projecting to the substantia nigra in the rat. J. Histochem. and Cytochem., (1992), 40, 889-892.


