**MONOCLONAL ANTI-CONJUGATED DOPAMINE ANTIBODY** (mouse)

**Data Sheet**

**Code number : AM001**

**Description**
Monoclonal antibody was obtained after BALB/c mouse immunisation with the conjugates: Dopamine-Gluteraldehyde-Carrier proteins and hybridization of spleen cells with the myeloma cell line SP2/O/Ag14. Ascite production was performed in BALB/c mice.

**Purification**
The ascitic fluid was purified by ammonium sulfate precipitation and gel filtration.

**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>L-DOPA-G-BSA</td>
<td>1/10,000</td>
</tr>
<tr>
<td>Tyrosine-G-BSA</td>
<td>1/36,000</td>
</tr>
<tr>
<td>Tyramine-G-BSA</td>
<td>1/&gt;100,000</td>
</tr>
<tr>
<td>Noradrenaline-G-BSA</td>
<td>1/&gt;100,000</td>
</tr>
<tr>
<td>Octopamine-G-BSA</td>
<td>1/&gt;100,000</td>
</tr>
<tr>
<td>Adrenaline-G-BSA</td>
<td>1/&gt;100,000</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1/&gt;100,000</td>
</tr>
</tbody>
</table>

(a) : Dopamine-G-BSA concentration/unconjugated or conjugated catecholamine concentration at half displacement;
(b) : Non-reduced conjugate;
G = Glutaraldehyde, BSA = Bovine Serum Albumine.

**Subclass** : IgG 1, Kappa.

**Recommended dilution**
The antibody was tested using the free floating PAP technique on rat dopaminergic areas. The anti-conjugated Dopamine antibody gave a good staining between a 1/5,000-1/20,000 dilution in these areas.

**Applications**
Immunohistochemistry, immunocytochemistry.

**Storage and handling**
Monoclonal antibody was lyophilized (dried freezed) with 0.01% merthiolate and was stable at +4°C. It must be reconstituted with 25µl or 50µl of distilled water (written on the bottle). Store the reconstituted antibody at +4°C.

**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) : 150-300ml/min
- Solution B (500ml) : 150-300ml/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 antibody**: The final dilution is 1/5,000 to 1/20,000 in solution C containing 0.1% triton X100, 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.

**PAP procedure**: Second antibody : Sections are incubated with 1/200 dilution of goat anti-mouse 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 mouse 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**