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# Mouse Anti-Conjugated Dopamine Monoclonal Antibody

**Catalog No:** MA2002

**Size:** 100 µl / vial

**Description:**
Monoclonal antibody was obtained after BALB/c mouse immunization with the conjugates : Dopamine-Glutaraldehyde-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.

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

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

| **Compound** | **Cross-reactivity ratio** [(a)] |
|---|---|
| Dopamine-G-BSA | 1 |
| L-DOPA-G-BSA | 1/10,000 |
| Tyrosine-G-BSA | 1/36,000 |
| Tyramine-G-BSA | 1/> 100,000 |
| Noradrenaline-G-BSA | 1/> 100,000 |
| Octopamine-G-BSA | 1/> 100,000 |
| Adrenaline-G-BSA | 1/> 100,000 |
| L-DOPA=G=BSA[(b)] | 1/> 100,000 |
| Dopamine | 1/> 100,000 |

(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.

**Immunohisto and cytochemical applications:**
*Detection of conjugated Dopamine in rat brain*

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**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.

N.B. : The antibody may be used at a higher dilution. The customer should explore the further antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer sys tem as used in our protocol may change the background and the antibody recognition.

**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.

**Detection:** Antibody-antigen complexes are detected using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of H2 O2 is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transfering sections in 5ml of Tris 0.05M.

## References

1. CHAGNAUD J.L., MONS N., TUFFET S., GRANDIER-VAZEILLES X. and GEFFARD M. Monoclonal antibodies against glutaraldehyde-conjugated dopamine. J. Neurochem., (1987) 49, 487-494 .

2. DECAVEL C., LESCAUDRON L., MONS N. and CALAS A. First visualization of dopaminergic neurons with a monoclonal antibody to dopamine : a light and electron microscopic study. J. Histochem. Cytochem., (1987) 35, 1245-1252.

3. SCHÜRMANN F.W., ELEKES K. and GEFFARD M. Dopamine-like immunoreactivity in the bee brain. Cell & Tissue Res., (1989), 256, 399-410.

4. GOLDMAN-RAKIC P.S., LERANTH C., WILLIAMS S.M., MONS N. and GEFFARD M. Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. Proc. Nat. Acad. Sci. USA, (1989), 86, 9015-9019.

**FOR RESEARCH USE ONLY. NOT FOR USE IN HUMAN DIAGNOTIC OR THERAPEUTIC PROCEDURES**.

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