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Monoclonal Antibody to Dopamine - Purified

Catalog No.: AM32615PU-N

Quantity: 0.1 mg

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM20P (for use in rat samples), AM03095PU-N

Clone: K56A

Immunogen: Dopamine-Gluteraldehyde-BSA.

Format:
State: Liquid purified IgG
Preservatives: 0.005% Merthiolate and 0.05% Sodium Azide
Stabilizers: 50% Glycerol

Applications:
Immunohistochemistry on Frozen Sections: 1/500-1/2,500 using free floating sections by the PAP technique on Rat dopaminergic areas.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This antibody recognizes Dopamine.
The cross-reactivities were determined using an ELISA test by competition experiments with the following compounds:
Compound : Cross-reactivity
Dopamine-G-BSA: 1
L-DOPA-G-BSA: 1/10,000
Tyrosine-G-BSA: 1/36,000
Tyramine-G-BSA: 1/›50,000
Noradrenaline-G-BSA: 1/›50,000
Octopamine-G-BSA: 1/›50,000
Adrenaline-G-BSA: 1/›50,000
Dopamine: 1/›50,000

G= Glutaraldehyde, BSA= Bovine Serum Albumin.

Species Reactivity: **Tested:** Rat.

Storage:
Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols:
For Dopamine Detection by Immunohisto/cytochemistry. Example for a Rat brain.
1. **SOLUTIONS TO BE PREPARED** - Solution must be prepared as needed.
Solution A: 0.1M cacodylate, 10g/L sodium metabisulfite, pH 6.2.
Solution B: 0.1M cacodylate, 10g/L sodium metabisulfite, 3-5% glutaraldehyde, pH 7.5.

2. **RAT PERFUSION** - The rat is anaesthetized with sodium pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with Solution A (30 mL): 150-300 mL/min, Solution B (500 mL): 150-300 mL/min.
3. **POST FIXATION:** 15 to 30 minutes in Solution B, then 4 soft washes in 0.05M Tris with 8.5 g/L sodium metabisulfite, pH 7.5 (Solution C).
4. **TISSUE SECTIONING:** Vibratome or cryostat sections can be used.
5. **REDUCTION STEP:** Sections are reduced with Solution C containing 0.1M sodium borohydride for 10 minutes. The sections are washed 4 times in solution C without sodium borohydride.
6. **APPLICATION OF DOPAMINE ANTIBODY:** Use a final dilution of 1:2,500-1:10,000 in Solution C containing 0.1% Triton X100 and 2% non-specific serum. Incubate 12 sections per 2 mL diluted antibody overnight, +4°C. Then wash the sections three times for 10 minutes each in Solution C. (Note that the antibody may be usable at a higher dilution. This should be explored to minimize the possibility of high background. Additionally, note that a change in the buffering system as indicated in the protocol may change the background and antibody recognition). The specific reaction is then revealed by PAP procedure.
7. **SECOND ANTIBODY:** Incubate the sections with a 1:50 to 1:200 dilution of goat anti-rabbit in Solution B containing 1% non-specific serum for either 3 hrs at 20°C or 2 hr at 37°C. Then wash the sections, 3 times, for 10 minutes each with Solution B.
8. **PAP:** Incubate the sections with the appropriate dilution of peroxidase anti-peroxidase (for free floating method) in Solution B containing 1% non-specific serum for 1-2 hours at 37°C. Then wash sections 3 times for 10 min each in solution B.
9. **VISUALIZATION:** The antigen-antibody complexes are visualized using DAB-4-HCl (25 mg/100 mL) (or other chromogen) in 0.05M Tris and filtrated; 0.05% hydrogen peroxide is added. Incubate the sections for 10 minutes at room temp. Stop the reaction by transferring the sections to 5 mL 0.05M Tris. Wash tissue with solution D using 2, 10 min washes. Mount sections on chrome-alum coated slides. Dry overnight at 37°C. Rehydrate sections using conventional histological procedures. Coverslip using rapid mounting media.