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Polyclonal Antibody to Tyramine - Serum

Catalog No.: AP32496SU-N

Quantity: 0.1 ml

Host: Rabbit

Immunogen: p-Tyramine-gluteraldehyde-N-alpha-acetyl-L-lysine-N-methylamide

Format:
State: Serum
Preservatives: 0.05% Sodium azide.

Applications:
Immunohistochemistry: 1/1000-1/2500
by PAP (see suggested protocol)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This antibody recognizes p-Thyramine.
The cross-reactivities were determined using either ELISA or RIA techniques, at concentration/unconjugated or conjugated amino acid concentration at half displacement.
Cross-reactivity ratio
Noradrenaline-G-BSA: 1
Octopamine-G-BSA: 1/30
Dopamine-G-BSA: 1/70
Adrenaline-G-BSA: 1/180
L-Dopa-G-BSA: 1/›5000
p-Tryamine-G-BSA: 1/›5000
Noradrenaline: 1/›5000
The antisera was also tested for specificity using the free-floating PAP technique on rat locus coeruleus. (Abbreviations: (BSA) = Bovine serum albumin, (G) = Glutaraldehyde)
Species: Rat.
Other species not tested.

Storage:
Upon receipt, store undiluted (in aliquots) at -20°C.
Avoid repeated freezing and thawing.
Shelf life: One year from despatch.

Product Citations:
Originator or purchased from resellers:
1. Parraguez VH, Atlagich MA, Urquieta B, Galleguillos M, De Los Reyes M, Kooyman DL, et al. Expression of vascular endothelial growth factor and endothelial nitric oxide synthase is increased in the placenta of sheep at high altitude in the Andes. Can J Vet Res. 2010 Jul;74(3):193-9. PubMed PMID: 20885843.
2. Tison F, Mons N, Geffard M, Henry P. The metabolism of exogenous L-dopa in the brain: an immunohistochemical study of its conversion to dopamine in non-catecholaminergic cells of the rat brain. J Neural Transm Park Dis Dement Sect. 1991;3(1):27-39. PubMed PMID: 2064729.

General Readings:

1. Selcho M, Pauls D, El Jundi B, Stocker RF, Thum AS. The role of octopamine and tyramine in Drosophila larval locomotion. J Comp Neurol. 2012 Nov 1;520(16):3764-85. doi: 10.1002/cne.23152. PubMed PMID: 22627970.
2. Crisp KM, Klukas KA, Gilchrist LS, Nartey AJ, Mesce KA. Distribution and development of dopamine- and octopamine-synthesizing neurons in the medicinal leech. J Comp Neurol. 2002 Jan 7;442(2):115-29. PubMed PMID: 11754166.
3. Mons N, Tison F, Geffard M. Existence of L-dopa immunoreactive neurons in the rat preoptic area and anterior hypothalamus. Neuroendocrinology. 1990 Apr;51(4):425-8. PubMed PMID: 2111889.
4. Geffard M, Seguela P, Heinrich-Rock AM. Antisera against catecholamines: specificity studies and physicochemical data for anti-dopamine and anti-p-tyramine antibodies. Mol Immunol. 1984 Jun;21(6):515-22. PubMed PMID: 6431267.

Protocols:

SAMPLE PROTOCOL
for Neurotransmitter Detection by Immunohistochemistry. (Example for a rat brain.)
1.SOLUTIONS TO BE PREPARED - Solution must be prepared as needed.
Note:Tris can be replaced by a 0.01M phosphate solution.
Solution A: 0.1 M cacodylate acid, 10 g/l sodium metabisulfite,pH 6.2.(*)
Solution B: 0.1 M cacodylate acid, 2.5-5% glutaraldehyde, 10 g/l sodium metabisulfite, pH 7.5.(*)
Solution C: 0.05 M Tris, 8.5 g/l sodium metabisulfite, pH 7.5.(*)
Solution D: 0.05 M Tris, 8.5 g/l sodium chloride pH 7.5.(*)
(*) Adjust pH with NaOH or HCl if necessary.
In the case of GLUTAMATE, Tris can be replaced by .0.1 M PBS in solutions C and D.
2. RAT ANESTHESIA
The rat is anaesthetized with sodium pentobarbital or chloral hydrate. The anesthesia is correct when: on its' back, rat doesn't return to it's side & light reaction occurs pinching the tail.
3. RAT PERFUSION
Open the animal's thorax and rapidly cannulate the aorta via the left ventricle. Cut the right atrium or ventricle to allow efflux of blood and perfusate. Clamp off the descending aorta. Perfuse intracardially
through the aorta, using either a multi-speed pump or a large syringe.
Solution A (30 ml): 200-300ml/min
Solution B (500 ml): 200-300 ml/min
Solutions A and B must be perfused through the rat brain continuously without flow stopping when changing solutions.
Indications of a good perfusion:
-Limbs are blanching. Ears are bleached and very white.
-Liver loses it's color and becomes very hard.
-When cutting the rat nose, glutaraldehyde must leak drop by drop.
-The brain must be dark-yellow and hard. (The color is homogeneous without any white blots).
Indications of a incorrect perfusion:
-All the above indications do not appear.
-Glutaraldehyde leaks by the mouth. Rat eyes are swollen.
4. POST FIXATION:
Cover rat brain with Solution B and let soak 30-120 minutes, then soft wash 4 times in Solution C.
5. TISSUE SECTIONING:
50 µm slices, preferably by the 'vibratome' technique, using Solution C.
6. REDUCTION STEP:
Sections are reduced with Solution C containing sodium borohydride (0.1M) for 10mn. Then

the sections are washed carefully 4 times with stock Solution C.
7. WASHING:
The sections are washed 3X (15 minutes each) in cold (4°C) Sol'n C, then incubated 1-1.5 hrs at room temp. in Sol'n C plus 3% of non-specific serum (normal goat serum).
8. PRIMARY ANTIBODY:
Use a final dilution of 1/500-1/2500 in Solution C containing 0.5% Triton X100 and 2% non-specific serum. Incubate 12 sections per 2 ml diluted antibody overnight, +2-8°C on a rocker. Then wash the sections three times for 10 minutes each in Solution D. (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.
9. SECOND ANTIBODY:
Incubate the sections with a 1:50 to 1:200 dilution of goat anti-rabbit in Solution D containing 1% non-specific serum for either 3 hrs at 20°C or 1 hr at 37°C on a rocker. Then wash the sections, 3 times, for 10 minutes each with Solution D.
10. PAP:
Incubate the sections with the appropriate dilution of peroxidase anti-peroxidase (for free floating method) in Solution D containing 1% non-specific serum for 1-2 hours at 37°C on a rocker. Then wash sections 4 times for 10 min each in solution D.
11. VISUALIZATION:
The antigen-antibody complexes are visualized using DAB-4-HCl (25 mg/100 ml) 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.