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# Polyclonal Antibody to Norepinephrine - Serum

**Catalog No.:** AP32495SU-N

**Quantity:** 0.1 ml

**Background:** The chemical compound norepinephrine (NC(8)O(3)H(11), also known as noradrenaline, is a catecholamine that functions as both a neurotransmitter and a hormone. It is released from the adrenal glands and also by certain neurons. It affects parts of the human brain where attention and impulsivity are controlled. This compound affects the fight-or-flight response, activating the sympathetic nervous system to directly increase heart rate, release energy from fat and increase muscle readiness.
The host of physiological changes activated by a stressful event are unleashed in part by activation of a nucleus in the brain stem called the locus ceruleus. This nucleus is the origin of most norepinephrine pathways in the brain. Neurons using norepinephrine as their neurotransmitter project bilaterally from the locus ceruleus along distinct pathways to the cerebral cortex, limbic system, and the spinal cord, among other projections.

**Host:** Rabbit

**Immunogen:** Noradrenaline-glutaraldehyde-BSA

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

**Applications:** **Immunohistochemistry**: 1/500-1/2500
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 Noradrenaline reacted with glutaraldehyde.
(Glutaraldehyde required in fixation for reactivity.)
Reactivity with free noradrenaline is very poor. 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:** Human and 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.

**General Readings:** 1. Schreiner S, Wimmer P, Groitl P, Chen SY, Blanchette P, Branton PE, et al. Adenovirus type 5 early region 1B 55K oncoprotein-dependent degradation of cellular factor Daxx is

required for efficient transformation of primary rodent cells. J Virol. 2011 Sep;85(17):8752-65. doi: 10.1128/JVI.00440-11. Epub 2011 Jun 22. PubMed PMID: 21697482.
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4. Karasawa N, Nagatsu I, Sakai K, Nagatsu T, Watanabe K, Onozuka M. Immunocytochemical study of catecholaminergic neurons in the senescence-accelerated mouse (SAM-P8) brain. J Neural Transm. 1997;104(11-12):1267-75. PubMed PMID: 9503272.
5. Sluka KA, Westlund KN. Spinal projections of the locus coeruleus and the nucleus subcoeruleus in the Harlan and the Sasco Sprague-Dawley rat. Brain Res. 1992 May 1;579(1):67-73. PubMed PMID: 1623408.
6. Herness S, Zhao FL, Kaya N, Lu SG, Shen T, Sun XD. Adrenergic signalling between rat taste receptor cells. J Physiol. 2002 Sep 1;543(Pt 2):601-14. PubMed PMID: 12205193.
7. Geffard M, Henrich-Rock AM, Dulluc J, Seguela P. Antisera against small neurotransmitter-like molecules. Neurochem Int. 1985;7(3):403-13. PubMed PMID: 20492941.

**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.