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Polyclonal Antibody to Tryptophan - Serum

Catalog No.: AP32758SU-N

Quantity: 0.1 ml

Host: Rabbit

Immunogen: Tryptophan-gluteraldehyde-Poly-lysine

Format:
State: Liquid Serum
Preservatives: 0.05% Sodium Azide

Applications:
Immunohistochemistry: 1/1,000-1/2,500 by PAP (See suggested Protocol).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
Recognizes Tryptophan.
The cross-reactivities were determined using an ELISA test by competition experiments with the following compounds:
Tryptophan-G-PL: 1
5-Hydroxytryptophan-G-PL: 0.01
5-Methoxytryptophan-G-PL: 0.004
Tryptophan=G=PL (non reduced): 0.002
Tryptophan: 0.0003
Tryptamine-G-PL: 0.00009
5-Methoxytryptamine-G-PL: 0.00002
5-Hydroxytryptamine-G-PL: 0.000004
The antisera was also tested for specificity using the free-floating PAP technique on Rat brain.
Abbreviations: (G) Glutaraldehyde, (=) Non-reduced conjugate, (PL) Poly-lysine.

Species Reactivity: **Tested:** Rat.

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

General Readings:
1. Geffard M, Dulluc J, Rock AM. Antisera against the indolealkylamines: tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-methoxytryptophan, and 5-methoxytryptamine tested by an enzyme-linked immunosorbent assay method. J Neurochem. 1985 Apr;44(4):1221-8. PubMed PMID: 3919158.
2. Seguela P, Geffard M, Buijs RM, Le Moal M. Antibodies against gamma-aminobutyric acid: specificity studies and immunocytochemical results. Proc Natl Acad Sci U S A. 1984 Jun;81(12):3888-92. PubMed PMID: 6587397.

Protocols:
Sample Protocol for Neurotransmitter Detection by Immunocytochemistry. 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 .01 M PBS in solutions C and D.

2. **RAT ANAESTHESIA** - The rat is anaesthetized with sodium pentobarbital or chloral hydrate. The anaesthesia 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): 150-300 mL/mn
Solution B (500 mL): 150 mL/mn

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 in cold (4 deg) 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 1:1,000-1:2,500 in Solution C containing 0.2% Triton X100 and 1% 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 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 2 hr at 37°C. 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. Then wash sections 3 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.