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Polyclonal Antibody to Histamine - Serum

Catalog No.: AP32418SU-N

Quantity: 0.1 ml

Host: Rabbit

Immunogen: Histamine conjugated to KLH with EDAC

Format:
State: Liquid Serum
Preservatives: 0.09% Sodium Azide

Applications:
Immunohistochemistry: 1/1,000-1/4,000.on brain and stomach using PAP or ABC. Recommended fixative is 4% EDAC (1-ethyl-3(3-dimethylaminoproply)-carbodiimide) or EDAC + PFA.
Immunocytochemistry: 1/200-1/5,000 on cultured cells (2H3 mastocytoma):
Note: The antibody is not suitable for use on samples fixed only with aldehydes.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Recognizes Histamine. No reactivity to L-Histadine containing peptides. Preabsorption of the antiserum with a Histamine-BSA or Histamine-Ovalbumin conjugate (1-100 mg/mL depending on tissue) completely abolished the specific reaction.

Species Reactivity: **Tested:** Vertebrates and Invertebrates (Human, Rat, Mouse, Frog, Fish, Mollusks, Flatworm).

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

General Readings:
1. Panula P, Yang HY, Costa E. Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci U S A. 1984 Apr;81(8):2572-6. PubMed PMID: 6371818.
2. Hakanson et al. Histochemistry (1986) 86:5-17.
3. Panula et al. J. Histoch Cytoch (1988) 36:259-269.
4. Panula, et al. Neuroscience (1990) 34:127-132.

Protocols:
Immunohistochemistry for Vertebrates
The histamine antibody can be applied to different species and tissues. The serum gives good results only when used on properly fixed tissue sections. Specific detection of histamine-like immunoreactivity requires the use of EDAC (1-ethyl-3(3-dimethylaminoproply)-carbodiimide) fixative. Immersion fixation is suitable for small animals and tissue blocks. Perfusion is recommended for vertebrates.
Solutions
1. 0.1 M sodium phosphate buffer, pH 7.4
2. 4% EDAC (1-ethyl-3(3-dimethylaminoproply)-carbodiimide) in 0.1M sodium phosphate buffer (pH 7.4); the final pH will be around 7.0. Always use freshly prepared solution.
3. 0.9% saline.

Application to small laboratory animals (Vertebrates)
1. Open the thorax under suitable deep anesthesia (e.g. pentobarbital). Open the right heart auricle with small scissors, Perfuse through the left ventricle or ascending aorta with saline until all blood is washed out (about 50 mL
in a rat).
2. Perfuse with fresh 4% EDAC through the same needle. Use about 1 mL/gram of body weight.
3. Remove the tissues and immerse them in 4% EDAC for 2 hours or overnight.
4. Wash the tissue in 0.1 M sodium phosphate buffer containing 20-30% sucrose overnight.
5. Freeze the tissues and make cryostat sections for immunohistochemistry.

Tissues from many species can be fixed by immersion in 4% EDAC overnight at 2-8°C. The pieces should be no thicker than 0.5 cm because the fixative does not pentrate very well. Note that the tissues are not firm hard after fixation with EDAC. Handling free floating sections requires experience, sections collected on adhesive-coated slides are easy to handle.

If aldehyde fixation is necessary for e.g double staining procedures, EDAC perfusion should be followed by perfusion with 4% paraformaldehyde in the same buffer (and if needed 0.5% glutaraldehyde + 0.2% saturated picric acid). However these samples must be treated with 0.5% borohydride after sectioning.

Incubation with the histamine antiserum (diluted in PBS with 0.25% Triton) is best performed at 2-8°C for 24-48 hours. Extended incubation times of up to 5 days have also been used successfully.

Immunocytochemistry for Cell Culture
The histamine antibody can be applied to primary cell cultures and cell lines from different species and tissues. The serum gives good results only when used on properly fixed tissue sections. Specific detection of histamine-like immunoreactivity requires the use of EDAC (1-ethyl-3(3-dimethylaminoproply)-carbodiimide) fixative. Immersion fixation is suitable for small animals and tissue blocks. Perfusion is recommended for vertebrates.

Solutions
1. 0.1 M sodium phosphate buffer, pH 7.4
2. 4% EDAC (1-ethyl-3(3-dimethylaminoproply)-carbodiimide) in 0.1M sodium phosphate buffer (pH 7.4); the final pH will be around 7.0. Always use freshly prepared solution.
3. 0.9% saline

Suggsested Immunochemistry Protocols (cont)
Incubation procedure
1. Pour out the culture medium and wash the cells with 0.9% saline or 0.1 M sodium phosphate buffer. (This can be omitted if the cells are sensitive to changes.)
2. Pour in freshly prepared 4% EDAC, fix at 2-8°C for 0.5-12 hours depending on the thickness of the culture.
3. Wash with 0.1 M sodium phosphate buffer or PBS 2 x 10 min.
4. Preincubate with normal serum from the species used for production of the secondary antiserum for 20 minutes at room temperature. Pour out the normal serum, do not wash.
5. Incubate with the histamine antiserum diluted appropriately (1:200-1:5,000) for 24-48 hours at 2-8°C.
If electron microscopy or double staining procedures with other antibodies are needed, the

cells can be fixed after 4% EDAC with a mixture of 4% paraformaldehyde, 0.5% glutaraldehyde and 0.2% saturated picric acid for 30 minutes. After thorough washing, these samples must be treated with 0.5% sodium borohydride (and washed extensively) before incubation.
Any routinely used detection method (indirect immunofluorescence, PAP procedure, biotin-streptavidin peroxidase) can be used.