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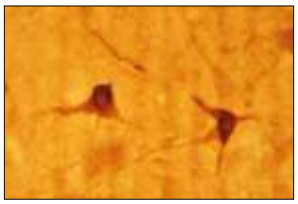
Antibodies that Shine



IHC image of neurons in the raphe nucleus of rat brainstem.

5-HIAA (5-Hydroxyindoleacetic Acid) Antibody

Catalog #	**24274**	**Product type**	Primary antibodies
Lot #	**1229001**	**Clonality**	Polyclonal
Form	Lyophilized whole serum (100 µL)	**Isotype**	IgG
Host	Rabbit	**Preservative**	≤ 0.09% sodium azide
Reacts With	Mollusca, Rat, Sea Slug	**Antigen**	5-HIAA coupled to bovine serum albumin (BSA) with paraformaldehyde.

INSTRUCTIONS

Preparation	Do not reconstitute until ready to use since the product is most stable when lyophilized. The product does not need to be kept cooled during shipping; however, for long-term storage, store lyophilized antibody until ready to use at -15ºC or lower. Reconstitute with 100 µL of distilled or deionized water. After reconstitution, use immediately or refrigerate at 2º–8ºC. To avoid freeze/thaw cycles, dilute unused antibody with PBS or Tris buffer at a dilution no higher than 1/10, then aliquot and freeze at -15ºC or lower. Refer to the Instruction Manual available online at www.immunostar.com for information on tissue preparation, immunostaining techniques, troubleshooting, and formulas.

APPLICATION

IHC Quality Control	The antibody produces significant labeling of raphe neurons in normal rat. In rats whose serotonergic system has been activated, staining intensity is increased to a significant label. Recommended dilutions of the antiserum are 1/200–1/400 for indirect immunofluorescence and 1/4,000–1/8,000 for biotin-streptavidin/HRP technique. The specificity of the antiserum was evaluated using a model system of gelatin-indole plugs by a method similar to published procedures (Schipper and Tilders, 1983). Results showed that the 5-HIAA antibody dose dependently stained 5-HIAA but did not stain any concentration of 5-HT or 5-HTP. The antiserum was also tested by preadsorption at 25 µg/mL with various BSA conjugates. While preadsorption with 5-HIAA conjugate completely eliminates immunolabeling, preadsorption with conjugates of 5-HT, 5-HTP and dopamine had no effect on staining intensity or distribution of stain.
Tissue	Rat dorsal and median raphe neuronal cell bodies. Serotonergic system may be activated by salt loading which is achieved by 2% NaCl placed in drinking water for 48 hours prior to perfusion.
Perfusion Fixation	• Fixation: 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4; 500 mL over 20 min. • Post Fixation: 1.5 hour at 4°C in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. • Note: Paraformaldehyde is a necessary component of fixation for this antiserum. If needed for other applications, glutaraldehyde may be used at low levels (0.1–0.3%) in conjunction with paraformaldehyde.
Sections	10 µm cryostat or 50 µm vibratome
Tissue Incubation	18–24 hours at 2°–8°C.
Detection System	Use IF or Bn-AV/HRP reagents at dilutions recommended by the manufacturers.
Suggested Dilution	1/4,000–1/8,000 in PBS/0.3% Triton X-100 – Bn-AV/HRP immunohistochemistry

NOTES

Special Instructions	It is recommended that the researcher perform a primary antibody dilution series using our dilution recommendations as a guideline. Note that a change in the fixation or buffering system from our protocol may change the configuration of the protein which could alter the reactivity with the tissue tested.
Concentration	Not applicable. Antibody concentration is only relevant for purified antibodies.
Journal References	www.immunostar.com/publications

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