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RABBIT ANTI-NEUROTENSIN POLYCLONAL ANTIBODY

CATALOG NUMBER:	AB5496	**QUANTITY:**	100 µg
LOT NUMBER:		**CONCENTRATION:**	1 mg/mL

ALTERNATE NAMES: NT

HOST/ISOTYPE: Rb IgG

BACKGROUND: Neurotensin is one of two peptides expressed from a common precursor gene. Neuromedin N being the other partner. Neurotensin is a secreted tridecapeptide, which is widely distributed throughout the central nervous system, and may function as a neurotransmitter or a neuromodulator. It may be involved in dopamine-associated pathophysiological events, in the maintenance of gut structure and function, and in the regulation of fat metabolism.

SPECIFICITY: Reacts with Neurotensin protein, no reactivity with Neuromedin N.

IMMUNOGEN: Purified Neurotensin conjugated to BSA

APPLICATIONS: Immunohistochemistry: 3-20 µg/mL see suggest protocol
ELISA: 2-5 µg/mL coating; 1:1000-1:10:000 detection

SPECIES REACTIVITY: Human, rat, mouse, bovine, other species not yet tested.

PRESENTATION: Liquid in PBS containing 1% BSA and 0.1% sodium azide as a preservative.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.

RELATED REFERENCES:

Uhl, GR, et al., *PNAS.USA* (1977) **74**:4059-4063.
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Woodhams, PL, et al., *Neuroscience* (1983) **8**:677-703.
Milner, TA, Pickel VM, *J Comp Neurol* (1986) **247**:326-343.
Mai, JK et al., *Neuroscience* (1987) **22**:499-524.
Williams, FG, Beitz, AJ, *J Histochem Cytochem* (1989) **37**:831-841.
Woulfe, J, Beaudet, A, *J Comp Neurol* (1992) **321**:163-176.
Dufourny, L, et al., *J Chem Neuroanat* (1999) **17**:33-43.

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APPLICATION NOTES FOR AB5496

IMMUNOHISTOCHEMISTRY

Anesthetize animals and perfuse them transcardially as follows:
a) Flush with cold (4°C) oxygenated Calcium free Tyrodes
b) Perfuse with 4% formaldehyde in 0.16M phosphate buffer at pH 6.9
c) Perfuse with 10% sucrose solution in 0.1M phosphate buffer (pH 7.2)

Sections
Cut 5-30 μm sections using a cryostat and mount them on subbed histological slides.

Immunofluorescence
Dilute Neurotensin antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.1% Triton X-100. Incubate sections for 24-48 hours at 2-8°C. Wash in PBS (three times for 10 minutes). Incubate for one hour or two hours at room temperature with properly diluted donkey anti-rabbit secondary antibody conjugated to a fluorescent probe such as FITC (Catalog Number AP182F), Rhodamine (Catalog Number AB182R) or Cy3 (Catalog Number AP182C). Wash in PBS (three times for 10 minutes) and mount with a PBS/glycerol solution containing 0.1% phenylenediamine to reduce fading. If not stained with FITC, sections can be dehydrated in grading alcohols (50%, 75%, 80%, 96% and 100%), cleared with xylene and mounted with DPX (Fluka, Ronkonkoma, NY). Staining can be visualized by using both conventional and confocal microscopy.

Indirect Avidin-Biotin Peroxidase
Incubate sections with 0.3% H_2O_2 in PBS for 15 minutes at room temperature to block endogenous peroxidase. Rinse sections with PBS (three times for 10 minutes). Dilute Neurotensin antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.1% Triton X-100. Incubate sections overnight at 2-8°C and then wash in PBS (three times for 10 minutes). Incubate sections with properly diluted biotinilated goat anti-rabbit secondary antibody (Catalog Number AP132B) diluted in PBS (do not add sodium azide!) for one hour at room temperature. Rinse sections three times for 15 minutes and incubate sections with horseradish peroxidase-streptavidin complex (Catalog Number SA108) properly diluted in PBS/0.1% Triton X-100 for 40 minutes at room temperature. Wash sections PBS and incubate them in substrate solutions (e.g. DAB, AEC, etc.) to achieve necessary intensity of Neurotensin staining.

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