NEUROMICS

Neurotensin Receptor 2 (NTS2) Datasheet

Levocabastine-Sensitive Neurotensin Receptor

Catalog Number:	RA17100	**Host:**	Rabbit
Product Type:	Polyclonal antiserum	**Species Reactivity:**	Rat
Immunogen Sequence:	WPPRPSPSAGLSLEA a.a. 7-21 of the rat NTS2 receptor	**Format:**	Whole Serum Sent in liquid form

Applications: Immunohistochemistry 1:1000
Immunocytochemistry 1:2000
Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted serum and immediately place at -20ºC. Serum may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20ºC. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

Immunocytochemistry
Transfected COS-7 cells, plated on poly-L-lysine–coated glass coverslips, were fixed for 20 minutes with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, rinsed with 0.1 M Trisma base-buffered saline (TBS), pH 7.4, and preincubated for 30 minutes at RT with a blocking solution consisting of 5% normal goat serum (NGS), 2% BSA, and 0.1% Triton X-100 in 0.1 M TBS. Cells were then incubated overnight at 4°C with the anti-NTS2 peptide antiserum diluted 1: 2,000 in 0.1 M TBS, pH 7.4, containing 1% NGS and 0.05% Triton X-100. After washing with TBS, cells were incubated for 1 hour at RT with appropriate secondary antibody, washed twice in TBS, and mounted on glass slides with Aquamount.

Immunohistochemistry
Adult male Sprague-Dawley rats (200–250 g) were anesthetized with sodium pentobarbital (Somnotol; 1.2 ml/kg) and perfused transaortically with a freshly prepared solution of 4% PFA in 0.1 M PB, pH 7.4. Brains were rapidly removed, cryoprotected overnight in 0.1 M PB containing 30% sucrose at 4°C, and frozen for 1 minute in isopentane at -40°C. SAections, 30 μm thick, were cut on a freezing microtome and collected in 0.1 M PB. Immunohistochemistry as performed according to the avidin biotinylated–HRP complex (ABC) method by using a Elite BC kit (Vector Laboratories, Burlingame, CA). Briefly, free-floating sections were washed twice with 0.1 M TBS, pH 7.4, pretreated for 30 minutes with 3% hydrogen peroxide in 0.1 M TBS to quench endogenous peroxidase. Sections were then washed twice with TBS (2 x 10 minutes), preincubated for 1 hour at RT in a blocking solution containing 3% NGS and 0.2% Triton X-100 in TBS, and incubated overnight at 4°C with the primary NTS2 antibody (1: 1,000) diluted in TBS containing 0.05% Triton X-100 and 0.5% NGS. After two rinses in TBS containing 1% NGS (2 x 10 minutes), sections were incubated for 1 hour at RT in biotinylated goat anti-rabbit immunoglobulin diluted 1:400 in TBS (Vector laboratories) and then 1 hour in Elite ABC solution (Vector; prepared according to the manufacturer's instructions). Visualization of bound peroxidase was achieved by reaction in a solution of 0.1MTris-HCl (TB; pH 7.4) containing 0.05% 3-3-diaminobenzidine (DAB, Sigma-Aldrich), 0.04% nickel chloride, and 0.001% H2O2. The DAB reaction was monitored under a microscope to determine the optimal duration of incubation (5 minutes maximum) for yielding intense immunolabeling with minimal background staining. This reaction was stopped by several washes with 0.1 M TB.

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