NEUROMICS

Endomorphin 1 and 2 Data Sheet

Catalog Number:	RA21002	**Host:**	Rabbit
Product Type:	Polyclonal antiserum	**Species Reactivity:**	Rat, Mouse, Human, Monkey, Pig

Introduction: This antiserum will cross react with both Endomorphin-1 (Tyr-Pro-Trp-Phe-NH2) and Endomorphin-2 (Tyr-Pro-Phe-Phe-NH2). Antiserum was raised in rabbits by using synthetic Endomorphin-2 peptide conjugated to BSA.

Sensitivity: Sensitivity has been determined by ELISA test. Less than 1 ng of antibodies could be detected after binding to Endomorphin-2 which is conjugated to titer plate.

Cross Reactivity:

Endomorphin-2	100
Endomorphin-1	70
Leu^5-Enkephalin	<0.01
Met^5-Enkephalin	<0.03
beta-Endorphin	<0.01

Storage and Handling: This product is a lyophilized affinity purified antiserum. It is recommended to store this antiserum at 0-4°C prior to reconstitution. It is recommended to reconstitute it with distilled or deionized water (to make 1 mg/ml concentrate) containing 0.1% of sodium azide or other preservative; divide into aliquots and store frozen at -20°C or lower. Avoid repeated freezing and thawing. Frozen aliquotes can be stored for at least six months. For better performance, once a frozen aliquot has been thawed, make a working dilution (antibody dilution buffer should contain 0.1% of sodium azide or other preservative) and keep it at 0-4°C (stable for at least 1 month). For slide-mounted tissue sections working dilution for immunofluorescence histochemistry is 3-10 g/ml, whereas for avidin-biotin immunohistochemistry antiserum may be diluted to 1 g/ml. For free floating tissues sections working dilutions should be determined in titration experiments.

Special Instructions: Optimal working dilution should be determined by individual investigator

Application Notes

Immunohistochemistry

Sections: Cut 5-30 m tissue sections by using a cryostat and mount them on subbed histological slides.
Immunofluorescence: Dilute Endomorphin-1 antibodies with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% bovine serum albumin and 0.01 Triton X-100. Incubate sections for 24-48 hours in a cold room. Wash in PBS (15 min x 3). Incubate for 1 or 2 hours at room temperature with donkey anti-rabbit secondary antibodies conjugated to fluorescent probes (FITC, LRSC; Cy 3, Cy 2, Cy5 - - Jackson ImmunoResearch Laboratories, Inc.,) or Alexa dyes (Molecular Probes). Wash in PBS (15 min x 3) and mount under coverslips using mediums reducing fading of fluorophores (e.g. SlowFade Molecular Probes). If stained using cyanin fluorophores, sections can be dehydrated in grading alcohols (50%, 75%, 80%, 96% and 100%), cleared in xylene and mounted with DPX (for reference see a catalogue of Fluka, Ronkonkoma, NY). Staining can be visualized by using both conventional and confocal microscopy.
Indirect immunostaining technique: Incubate sections with 0.3% H2O2 in PBS for 15 minutes at room temperature to block endogenous peroxidase. Rinse sections with PBS (three times for 10 minutes), incubate sections overnight at +4oC and then wash in PBS (three times for 10 minutes). Incubate sections with biotinilated goat anti-rabbit secondary antibodies diluted in accordance with manufacturers recommendations in PBS (do not add sodium azide!) for 1 hour at room temperature, rinse sections three times for 15 minutes and incubate sections with ABC reagent (Vector Laboratories) at room temperature for 30 minutes. Rinse sections in PBS and incubate them in substrate solutions (e.g. DAB, AEC or VIP - - Vector Laboratories) to achieve necessary intensity of Endomorphin-1 staining.

FOR RESEARCH USE ONLY

www.neuromics.com

Neuromics Antibodies • 210 Orchard St N • Northfield, MN 55057
phone 507-645-8020 • fax 612-677-3976 • e-mail pshuster@neuromics1.com