

Endomorphin 1

Data Sheet

Catalog Number:	RA21001	Host:	Rabbit
Product Type:	Affinity purified polyclonal antiserum	Species Reactivity:	Rat, Mouse, Human, Monkey, Pig
Introduction:	Antiserum to Endomorphin-1 (Tyr-Pro-Trp-Phe-NH ₂), was raised in rabbits by using synthetic Endomorphin-1 peptide conjugated to BSA. Selectivity towards Endomorphin-1 has been achieved by separating fraction of affinity purified antibodies which did not bind to Endomorphin-2.		
Sensitivity:	Sensitivity has been determined by ELISA test. Less than 1 ng of antibodies could be detected after binding to Endomorphin-1 which is conjugated to titer plate.		
Cross Reactivity:	Endomorphin-1	100	
	Endomorphin-2	5	
	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. 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 aliquots 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, recommended working dilution for immunofluorescence histochemistry is 15 mg/ml, whereas for avidin-biotin immunohistochemistry antiserum may be diluted to 5 mg/ml.

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

Application Notes

Immunohistochemistry

Sections: Cut 5-30 µm 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 +4°C and then wash in PBS (three times for 10 minutes). Incubate sections with biotinylated 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.

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