NEUROMICS

Endomorphin-2 Data Sheet

Catalog Number:	RA10111	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Rat, Primate
Immunogen Sequence:	YPFF	**Format:**	50 ug Lyophilized from 10 mM PBS

Applications: Immunohistochemistry: 1:150-1:200
Immunocytochemistry: 1:100-1:200

Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage and Handling: 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

Cross-reactivity (%)

Endomorphin-2	100
Endomorphin-1	3
Leu5-Enkephalin	<0.01
Met5-Enkephalin	<0.03
beta-Endorphin	<0.01

Immunohistochemistry: Antibody was used on perfusion fixed tissue. Perfusion: 1) calcium-free Tyrode's solution, 2) paraformaldehyde-picric acid fixative, and 3) 10% sucrose in PBS as a cryo-protectant. Desired tissues were dissected and stored overnight in 10% sucrose in PBS.

Slide-mounted tissue sections were processed for indirect immunofluorescence. Slides were incubated with blocking buffer for 1 hour at room temperature. Primary antiserum was diluted with blocking buffer to the appropriate working concentration. Blocking buffer was removed and slides were incubated for 18-24 hours at 4ºC with primary antiserum. Slides were rinsed 3 times and then incubated with secondary antibodies for 1 hour at room temperature. Slides were again rinsed 3 times and coverslipped. Staining was examined using fluorescence microscopy.
Note: Sodium azide (NaN3) interferes with peroxidase reactions and should not be used with peroxidase methodologies. If sodium azide is present in any steps of the staining procedure, the tissue should thoroughly be rinsed with sodium azide-free buffer before performing the peroxidase reaction.

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

Sections: Cut 5-30 um tissue sections by using a cryostat and mount them on subbed histological slides.
Immunofluorescence: Dilute Endomorphin-2 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.

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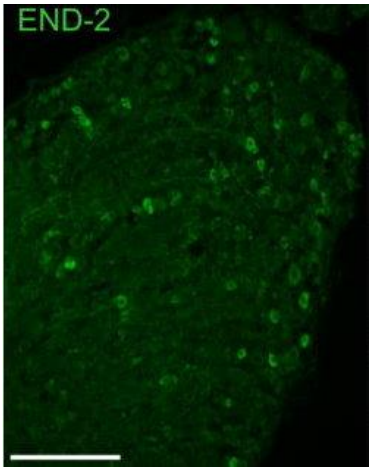
Indirect immunostaining technique: Incubate sections with 0.3% H2O2 in PBS for 15 minutes at room temperature to block endogenous peroxidase. Rinse sections with PBS (three times for 10 minutes), incubate sections overnight at +4oC and then wash in PBS (three times for 10 minutes). Incubate sections 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-2 staining.

Image: END-2 staining of neurons from neonatal rat lumbar (L4/5) DRG. doi: 10.1111/j.1460-9568.2008.06238.x.



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