NEUROMICS

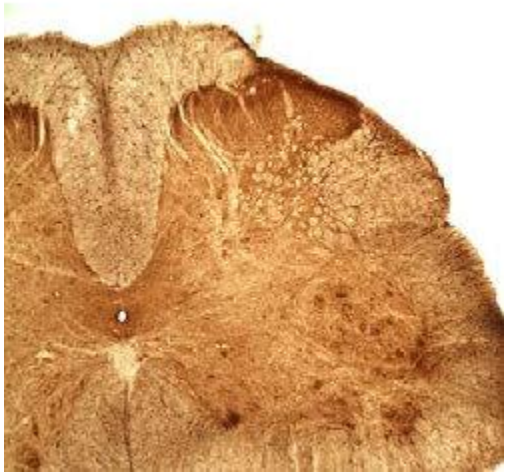
Mu Opioid Receptor Data Sheet

Catalog Number:	GP10106	**Host:**	Guinea pig
Product Type:	Whole serum	**Species Reactivity:**	Human, Rat, Mouse, Primate
Immunogen Sequence:	NHQLENLEAETAPLP Corresponding to residues 384-398 of the carboxy-terminus of rat mu opioid receptor	**Format:**	Whole Serum (with 0.05% sodium azide) Sent in liquid form
Applications:	Immunohistochemistry 1:100-1:400 Immunocytochemistry 1:100-1:400 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Storage: Maintain at +2-8ºC for 3 months or at -20ºC for longer periods. Stable for 1 year. Avoid repeated freeze-thaw cycles.		

Application Notes

Immunohistochemistry:

Image: MOR staining of Rat Dorsal Horn. Courtesy of Dr. Louis Gendron, University of Sherbrooke.



Protocol:

- Rats were deeply anesthetized with isoflurane and perfused through the aortic arch with 100 ml of heparin (75 U/ml of heparin in 0.9% saline) followed by 100 ml of a mixture of 3.75% acrolein and 2% PFA in 0.1 M PB, pH 7.4, and then by 300 ml of 2% PFA in the same buffer at 45 ml/min. Lumbar spinal cord was removed and postfixed in 2% PFA in 0.1 M PB for 1 h at 4°C. Sections (50 µm thick) were cut using a Vibratome and processed for MOR labeling.
- Sections were incubated in 1% sodium borohydride for 30 min and extensively rinsed in 0.1 M PB. They were then cryoprotected for 30 min in a solution consisting of 25% sucrose and 3% glycerol in 0.05 M PB and snap frozen with isopentane (–50°C) followed by liquid nitrogen.
- After being rapidly thawed in 0.1 M PB, sections were rinsed with TBS 0.1M and preincubated for 1 h at room temperature in 3% NGS diluted in TBS. They were then incubated for 36 h at 4°C in MOR antiserum diluted 1/500 in TBS containing 0.5% NGS. Sections were then rinsed twice with TBS and incubated for 1 h at room temperature with biotinylated anti-guinea pig antibody (1/400; Vector Laboratories).

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- Following three 10 min washes in TBS, sections were incubated 30 min with Vectastain Elite ABC (Vector Laboratories). Sections were rinsed three times with TBS and peroxidase complex revealed for 8 minutes with DAB substrate (2.2 mg/10 ml + 0.01% H_2O_2).
- At the end of this incubation, sections were washed twice with TBS, mounted on microscope slides, and dehydrated with ethanol.

Immunocytochemistry:
Mu opioid receptor transfected cells were processed for indirect immunofluorescence. Media was removed and cells were gently washed 3 times with serum-free media. Media was removed and cells were gently washed 3 times with serum-free media.

Following fixation process, 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.

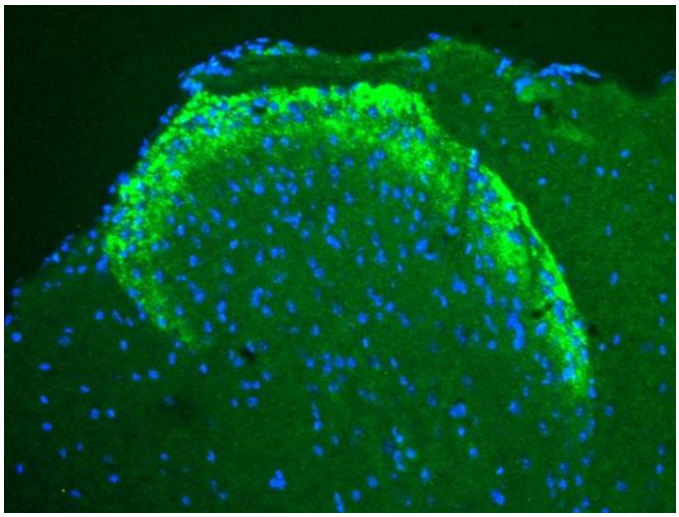

Image: MOR staining of Mouse Dorsal Horn, using a 1:50 dilution of 1:50.

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