NEUROMICS

GP10106, Mu Opioid Receptor, IHC Protocol:

1. 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.m 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.

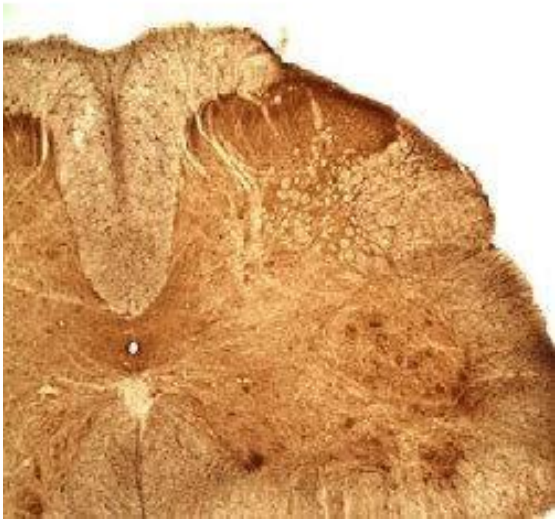
2. Sections were incubated in 1% sodium borohydride for 30 min and extensively rinsed in 0.1 M PB. They were then cryoprotected for 30 min in a solution consisting of 25% sucrose and 3% glycerol in 0.05 M PB and snap frozen with isopentane (–50°C) followed by liquid nitrogen.

3. 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).

4. 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% H2O2).

5. At the end of this incubation, sections were washed twice with TBS, mounted on microscope slides, and dehydrated with ethanol.

Image and protocol Courtesy of Dr. Louis Gendron, University of Sherbrooke.



Neuromics • 5325 West 74th Street, Suite 8 • Edina, MN 55439
Phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com