NEUROMICS

Mu Opioid Receptor Data Sheet

Catalog Number:	RA14138	**Host:**	Rabbit
Product Type:	Whole Serum	**Species Reactivity:**	Rat
Immunogen Sequence:	A synthetic peptide sequence corresponding to amino acids 384-398 predicted from the cloned rat MOR1. The peptide was conjugated to bovine thyroglobulin with glutaraldehyde.	**Format:**	Lyophilized. Contains less than 0.09% Sodium Azide as a Preservative.

Applications: Immunohistochemistry: 1:500-1:1000 Triton X-100 -Cy3 Fluorochrome
1:6000-1:10000 Triton X-100 -HRP Technique
Western Blot: Single Band at 65 kDa in cultured trigeminal ganglion neurons.

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Note that a change in the fixation or buffering system as used in our protocol may change the configuration of the protein and, therefore, may alter the reactivity with the tissue tested

Storage and Preparation: Reconstitute vial with 100 µL of distilled or deionized water.
Storage after reconstitution: Dilute with phosphate buffer or Tris buffer at dilutions no higher than 1/10, aliquot and freeze at -15° C or lower. Stability after reconstitution: Antibody can be stored for up to six months if handled as described above.

Application Notes

Mu Opioid Receptor antiserum was quality control tested using standard immunohistochemical methods. The antiserum demonstrates significant labeling of rat caudate putamen and spinal cord (dorsal horn) using indirect immunofluorescent and biotin/avidin-HRP techniques. Preadsorption with MOR peptide (384-398) at 10 µg/ml completely eliminates labeling. The specificity of the antiserum was determined by immunolabeling of transfected cells, Western Blot analysis and immunoisolation studies.

Immunohistochemistry:
Tissue-10-20 µm cryostat sections.
Fixative-4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4; 500 mL over ~ 20-30 min.
Post Fixation-1.5 hours at 4° C in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4
Tissue Incubation-18-24 hr at 2-8° C
Detection: Use biotin/avidin-HRP and Cy3 reagents at dilutions recommended by the manufacturers.

Description/Data:
Pleased note this antibody was generated using a synthetic peptide sequence corresponding to amino acids 384-398 predicted from the cloned rat MOR1. The peptide was conjugated to bovine thyroglobulin with glutaraldehyde (Mu Opioid Receptor Catalog#RA10104 was made using a peptide corresponding to amino acids 386-400 (NHQLENLEAETAPLP).
Three types of opioid receptors have been cloned -- mu, delta, and kappa. Opioid receptors are seven transmembrane G-protein coupled receptors. They share a high degree of homology and are most divergent at the N- and C-termini. Activation of mu opioid receptors leads to a decrease in neuronal excitability.

Western Blot:

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Sample Protocol- Lysates from TG cultures were prepared by adding 0.3 ml of PBS-TDS lysis buffer (PBS (10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4), 1 % Triton X-100, 0.1 M deoxycholate and 1% SDS) and incubating for 20 min on ice. Cell lysis was verified by microscopy. The lysate was triturated briefly through a 21 g needle, aliquoted and stored at −80°C. Forty microliters of the lysate was mixed with 20 µl of sample buffer and subjected to SDS-PAGE/Western blots analysis. Samples were loaded on a 15% gradient gel (BioRad) and transferred to PVDF Immobilon membranes (BioRad). Membranes were then blocked in 5% non-fat milk in Tris-buffered saline with 0.1% Tween (TBST) for 1 h at room temperature. Anti-β1 integrin or anti-MOR antibodies were incubated with the membrane at 4°C overnight. Then, the membrane was incubated at room temperature for 1 h with peroxidase-linked species-specific anti-IgG antibodies. Following incubation with a chemiluminescent substrate, the bands were visualized by ECL (Amersham).

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