# Mu Opioid (Catalog#: RA10104) Western Blot Protocol

*Please note: Bands for this protein are expected to be 70-80 Kd by western blotting analysis*

**Opioid agonist and antagonist treatment differentially regulates immunoreactive A-opioid receptors and dynamin-2 in vivo** *European Journal of Pharmacology 498 (2004) 87– 96*

Byron C. Yoburn*, Vishal Purohit, Kaushal Patel, Qiuyu Zhang

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway, Queens, NY 11439, USA

1. Individual spinal cords (n=5/group) were rapidly removed on ice and homogenized (Brinkman Polytron Homogenizer 20,000 rpm, 45 s) in 750 Al of ice-cold 50 mM Tris buffer (pH 7.4) and centrifuged at 12,000 rpm (2–4°C) for 20 min. Supernatant was discarded and the pellet was resuspended in 200 Al of lysis buffer (10% SDS (sodium dodecyl sulfate), 1 mM sodiumorthovandate, 12.5mM Tris, pH 7.4).

2. Samples were boiled for 15 min and centrifuged again at 12,000 rpm (2–4°C) for 60 min. The supernatant was collected for analysis and determination of protein concentration. Samples were diluted with an equal volume of sample buffer (10% SDS, 1% h-mercaptoethanol, 20% glycerol, 12.5 mM Tris base, bromophenol blue dye).

3. An aliquot of diluted sample from one spinal cord was loaded on each gel lane (15–25 Ag protein), so that 10 individual spinal cords (5 cords/treatment; 1 cord/lane) and a standard curve (see below) were loaded onto each polyacrylamide gel (Pager Gels 10% Tris–Glycine, Cambrex Bioscience, Rockland, ME).

4. Samples were separated by electrophoresis (0.02 amp for 85 min) and protein transferred to Immobilon-P PVDF (polyvinylidene difluoride) membranes (Millipore, Bedford, MA) using the miniprotean II (Bio-Rad) at 85 V for 100 min. Nonspecific binding sites on the membrane were blocked by overnight incubation (4°C) in blocking buffer (0.2% Aurora Blocking Reagent (ICN Biomedicals, Aurora, OH),in phosphate buffered saline (0.058M Na2HPO4, 0.017M NaH2PO4,0.068M NaCl); 0.05% Tween-20, Sigma, St. Louis, MO).

5. Membranes were then incubated (4 h, 25°C) with primary antibody (mu-opioid receptor, 1:500; Neuromics, Northfield, MN; Actin, 1:300; Santa Cruz Biotechnology, Santa Cruz, CA) in blocking buffer. Membranes were washed thrice with blocking buffer and then incubated (1 h, 25°C) with secondary antibody (for A-opioid receptor: Goat Antirabbit Immunoglobulin G - alkaline phosphatase (IgG-AP) (1:5000), ICN Biomedicals, Costa Mesa, CA; for Actin: Donkey Antigoat IgG-AP(1:5000) Santa Cruz Biotechnology).

6. Membranes were then washed thrice with blocking buffer, followed by two quick rinses with Assay buffer (20 mM Tris HCl, pH 9.8, 1 mM MgCl2). Bands were visualized using an alkaline phosphatase chemiluminescence assay (CDP Star Substrate, Novagen, Madison, WI).

7. A standard curve using increasing amounts of spinal cord protein from control mice (10–40 Ag/lane) was included on every gel. All data are converted into standard curve equivalents prior to analysis. Each experiment was repeated two to three times. Finally, in a series of control experiments, the selectivity of the primary antibody for the Mu-opioid receptor was assessed.

8. Briefly, tissues in which there was little or no specific binding for [3H] DAMGO (heart, lung, cerebellum, kidney, liver) were negative for immunoreactive Mu-opioid receptor. In addition, GH3 cells expressing the opioid receptor or the mu-opioid receptor were analyzed using the Western blot protocol. There were no specific bands in samples from GH3 cells expressing opioid receptors, whereas a single broad band was observed for samples from GH3 cells expressing mu-opioid receptors.The GH3 cells were a generous gift of Dr. Paul Prather (University of Arkansas Medical Center). Finally, preincubation of theprimary antibody with blocking peptide significantly reduced band density.