

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN μ (μ) OPIOID RECEPTOR

CATALOG NUMBER: HTS101M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND: Opiates derived from the opium poppy, *Papaver somniferum*, have been used in for millenia for their anti-diarrheal, analgesic and euphoric properties. More recently, endogenous peptides, enkephalins, dynorphins, and endorphins, were found to bind to the same sites as opiate alkaloids. The receptors for the classical opioids are three related GPCRs, μ , κ , and δ , that activate G_i/o to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor (Dhawan *et al.*, 1996). Chemicon's μ opioid receptor membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of μ opioid receptor interactions with its ligands. The membrane preparations exhibit an EC50 of 1.6nM for DAMGO in a GTP γ S binding assay.

APPLICATIONS: GTP γ S Binding and Radioligand Binding Assay.

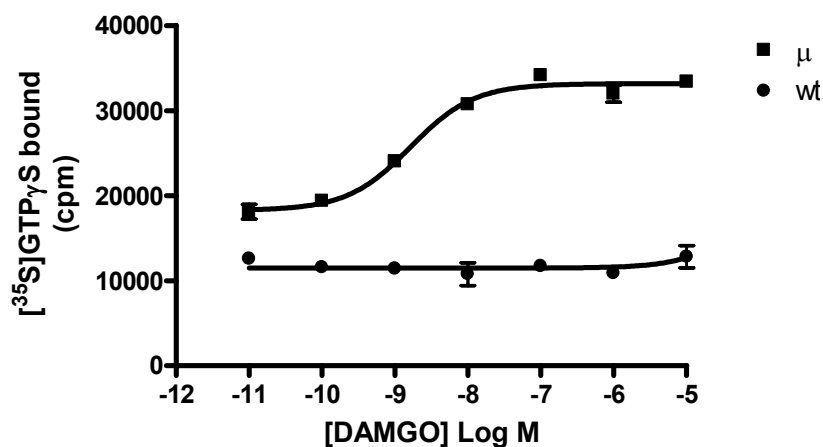


Figure 1. Binding of [³⁵S]-GTP γ S to μ opioid receptor membrane preparation. 5 μ g/well μ opioid receptor Membrane Preparation (catalog # HTS101M) and wild-type (wt) Chem-1 membrane preparation were incubated with 0.3 nM [³⁵S]-GTP γ S and increasing amounts of unlabeled DAMGO. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: EC50 in GTP γ S binding assay by DAMGO: ~ 1.6 nM

TRANSFECTION: full-length human OPRM1 cDNA encoding Mu (Accession number NM_000914)

HOST CELLS: Chem-5, an adherent cell line expressing a promiscuous G-protein.

ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [³⁵S]-GTP γ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl₂/0.5 μ M GDP in a nonbinding 96-well plate. Unlabeled DAMGO added to the final concentration indicated in Figure 1 (final volume 100 μ L), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (Millipore MAHF B1H) previously prewetted with water, and washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4. The plate is dried and counted.

One vial contains enough membranes for at least 200 assays (units), where one unit is the amount of membrane that will yield greater than 1000 cpm specific DAMGO-stimulated [³⁵S]-GTP γ S binding.

The μ opioid receptor membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Dhawan BN *et al.* (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48: 567-92.

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