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PRODUCT DATASHEET

ChemiScreen™ μ (Mu) Opioid Membrane Preparation

CATALOG NUMBER:	HTS101M	**QUANTITY:**	200 units
LOT NUMBER:	SC20170726	**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 Gi/o to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor (Dhawan *et al.*, 1996). 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 10.4nM for DAMGO in a GTPγS binding assay.

APPLICATIONS: GTPγS Binding Assay

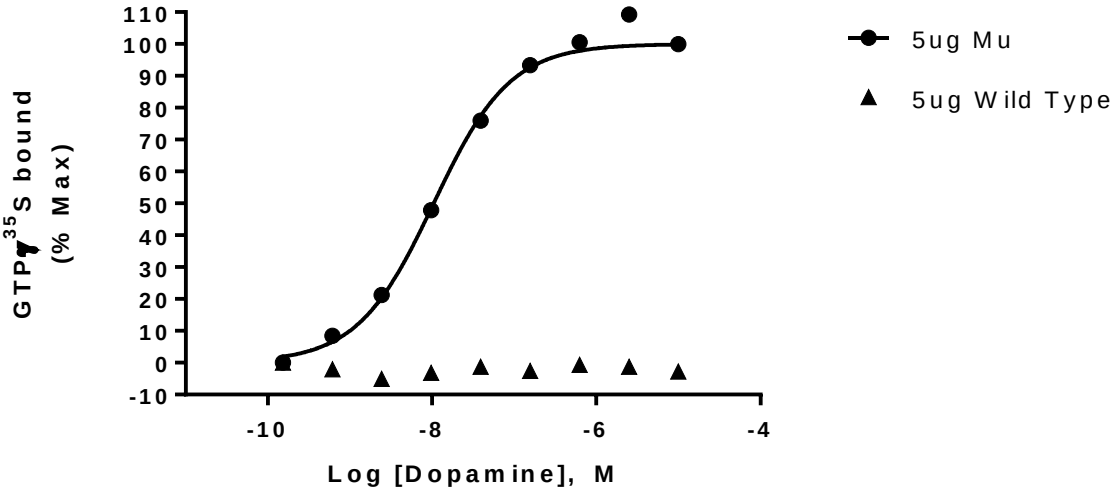

Figure 1. Binding of [^{35}S]-GTPγS to μ opioid receptor membrane preparation. 5 μg/well μ opioid receptor Membrane Preparation (catalog # HTS101M) and wild-type (wt) Chem-1 membrane preparation (catalog # HTS000MC1) were incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled DAMGO. Bound radioactivity was determined by filtration and scintillation counting. Representative sample data.

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SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTPγS binding assay by DAMGO: 10.4nM

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.

RECOMMENDED ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 µM GDP in a nonbinding 96-well plate. Unlabeled DAMGO added to the final concentration indicated in Figure 1 (final volume 100 µL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (EMD 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 [^{35}S]-GTPγS 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: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

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