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PRODUCT DATASHEET

ChemiScreen™ M_2 Muscarinic Acetylcholine Membrane Preparation

CATALOG NUMBER:	HTS115M	**QUANTITY:**	200 units
LOT NUMBER:	SC20180621	**VOLUME/CONCENTRATION:**	1 mL, 2.0 mg/mL

BACKGROUND:

The muscarinic acetylcholine receptor (mAChR) family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The M_1, M_3 and M_5 receptors couple to G_q to mobilize intracellular calcium, whereas the M_2 and M_4 receptors couple to $G_{i/o}$ to inhibit cAMP production (Caulfield and Birdsall, 1998). In urinary bladder trachea and stomach, M_2 augments the function of M_3 in promoting contractility, and activation of M_2 serves to counteract relaxation induced by increased cAMP levels (Ehlert et al., 2005; Wess, 2004). In addition, the ability of mAChR agonists to decrease heart rate appears to be mediated primarily by M_2. Agonists of mAChRs induce tremor, hypothermia, corticosterone release, and analgesia; each of these functions is mediated at least in part by M_2 (Wess, 2004). The M_2 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 M_2 interactions with its ligands. The membrane preparations exhibit a Kd of 0.76 nM for [^{3}H]-Scopolamine methyl chloride (NMS). With 10 µg/well M_2 Membrane Prep and 0.5 nM [^{3}H]-NMS, greater than 3-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

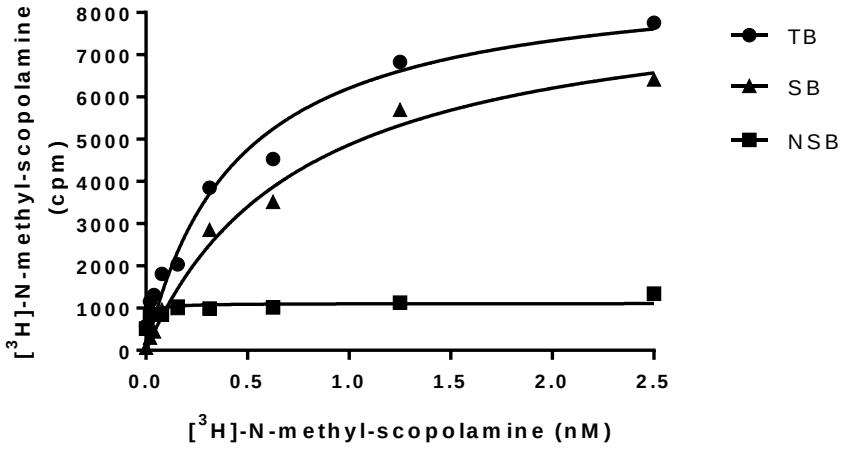

Figure 1. Saturation binding for M_2. 10 µg/well M_2 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled NMS in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Atropine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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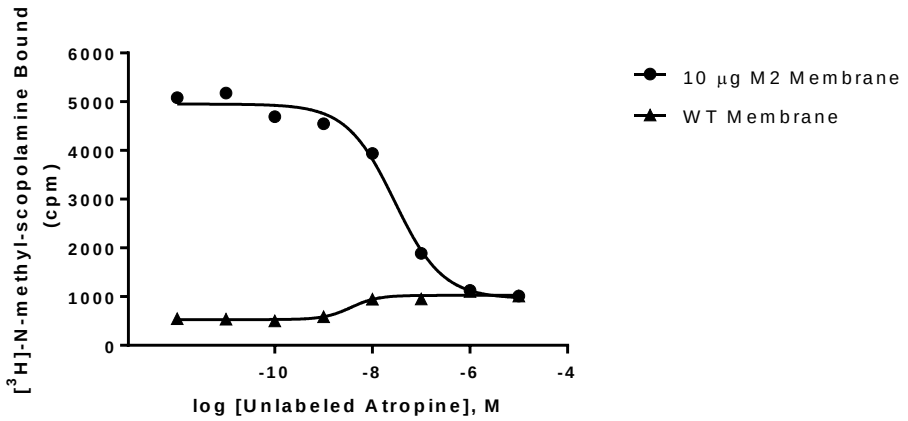

Figure 2. Competition binding for M_2: 10 µg/well M_2 Membrane Preparation (HTS115M) or 10 µg/well Chem-1 wild-type membrane (HTS000MC1) were incubated with 0.5 nM ^{3}H-labeled NMS and increasing concentrations of unlabeled Atropine. More than 3- fold signal:background ratio was obtained. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]- N-methyl-scopolamine binding: 9.65 pmol/mg protein
K_d for [^{3}H]- N-methyl-scopolamine binding: ~0.76 nM
Signal:Background: >3-fold

Species: Full-length human CHRM2 cDNA encoding M_2 (Accession Number: NM_000739)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous M_2 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 4mM Na_2HPO_4, 1mM KH_2PO_4, pH7.4. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 4mM Na_2HPO_4, 1mM KH_2PO_4, pH7.4, filtered and stored at 4°C.

Radioligand: [^{3}H] NMS (Perkin Elmer#:NEX-636)

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 3-fold signal:background with ^{3}H-labeled N-methyl-scopolamine at 0.5 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membranes 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. Caulfield MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.

2. Ehlert FJ *et al.* (2005) The M_2 muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder. *J. Pharmacol. Exp. Ther.* 313: 368-378.

3. Wess J (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.