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PRODUCT DATASHEET

ChemiScreen™ M_3 Muscarinic Acetylcholine Membrane Preparation

CATALOG NUMBER:	HTS116M	**QUANTITY:**	200 units
LOT NUMBER:	SC20180830	**VOLUME/CONCENTRATION:**	1 mL, 2 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). M_3 is expressed prominently in smooth muscle, and plays a primary role in mediating mAChR agonist-induced contractility. Mice lacking M_3 have dilated pupils, which indicates a role for M_3 in regulating tone of the pupillary sphincter muscle. In addition, M_3 plays a role in feeding, as indicated by the lean and hypophagic phenotype of M_3-null mice (Wess, 2004). M_3 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 antagonists of M_3 interactions with 4-DAMP. The membrane preparations exhibit a Kd of 0.49 nM for [^{3}H]-4-DAMP. With 10 µg/well M_3 Membrane Prep and 0.75 nM [^{3}H]-4-DAMP, a greater than 2.5-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

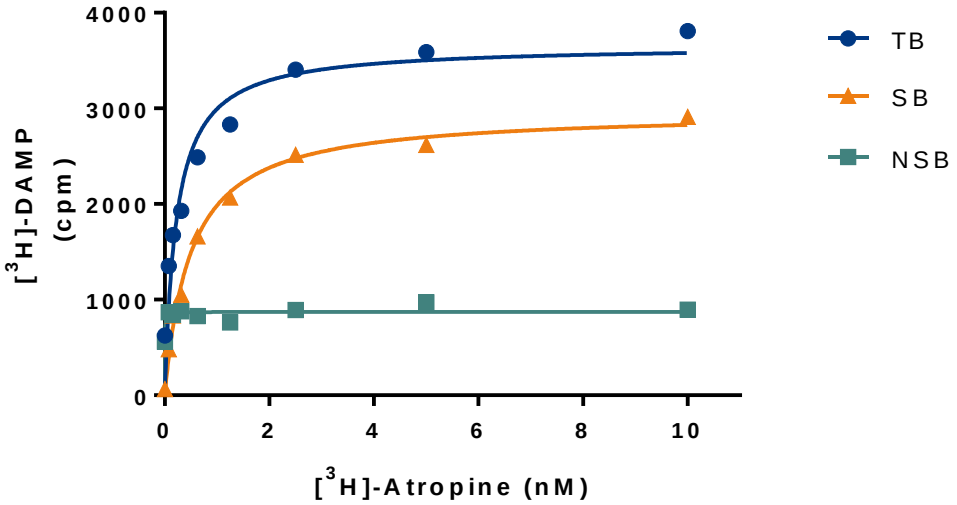

Figure 1. Saturation binding for M_3. 10 µg/well M_3 Membrane Preparation was incubated with increasing amount of [^{3}H]-4-DAMP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled atropine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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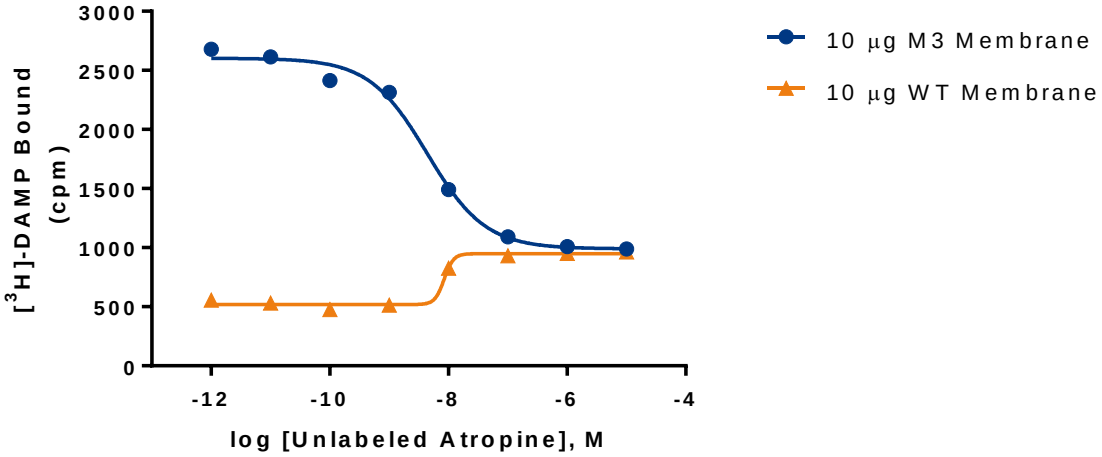

Figure 2. Competition binding for M_3. M_3 Membrane Preparation (10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.75 nM [^{3}H]-4-DAMP and increasing concentrations of unlabeled atropine, and more than 2.5- fold signal:background was obtained. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 3.22 pmol/mg protein
$K_{d:}$ ~0.49 nM
Signal:background: >2.5-fold

TRANSFECTION: Full-length human CHRM3 cDNA encoding M_3 (Accession Number: NM_000740)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous M_3 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, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. 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: 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{3}H] 4-DAMP (Perkin Elmer # NET1040)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 2.5-fold signal:background with ^{3}H-labeled 4-DAMP.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80^{0}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. Wess J (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.