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PRODUCT DATASHEET

ChemiScreen™ M_1 Muscarinic Acetylcholine Membrane Preparation

CATALOG NUMBER:	HTS044M	**QUANTITY:**	200 units
LOT NUMBER:	DAM1843099	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

The muscarinic acetylcholine receptor family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The M_1 receptor, along with the M_3 and M_5 receptors, signal through $G_{q/11}$ and subsequent release of Ca^{++} from the ER. The M_1 receptor is expressed in ganglia and mediates depolarization of ganglia by inhibition of voltage-gated M-type K^+ channels. In addition, the M_1 receptor mediates venous contraction (Caulfield and Birdsall, 1998). M_1 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_1 and its ligands. The membrane preparations exhibit a Kd of 1.4 nM for [^{3}H]-Pirenzepine. With 2 nM [^{3}H]-Pirenzepine, 10 μg/well M_1 Membrane Prep yields greater than 5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

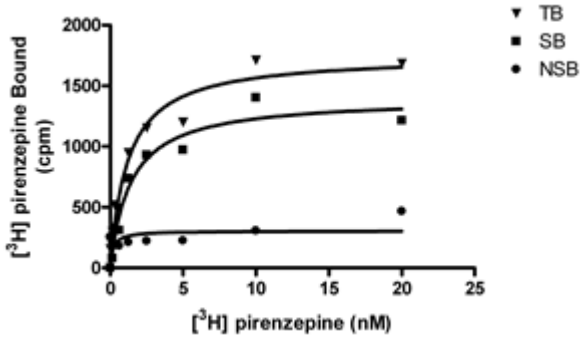

Figure 1. Saturation binding for M_1. 2.5 μg/well M_1 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled Pirenzepine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Pirenzepine. Bound radioactivity was determined by filtration binding and scintillation counting. Specific binding (SB) was determined by subtracting NSB from TB.

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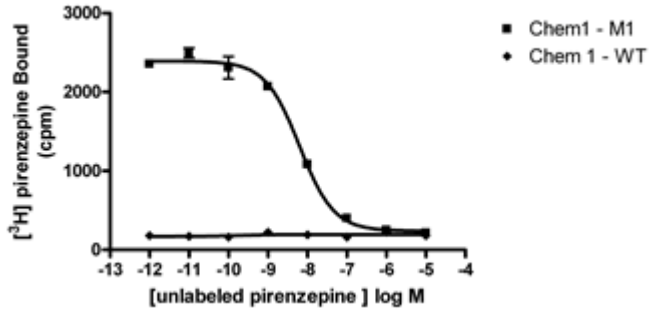

Figure 2. Competition binding for M_1. 10 µg/well M_1 Membrane Preparation and Wild-Type Chem-1 Membrane Preparation (catalog #HTS000MC1) were incubated with 2 nM ^{3}H-labeled Pirenzepine and increasing concentrations of unlabeled Pirenzepine.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with M_1 membrane preparation.

	10 µg/well
Signal:background	10.0
Specific binding	2152

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 4.9 pmol/mg
K_d: 1.4 nM

Species: Full-length human CHRM1 cDNA (Accession Number: NM_000738)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous M_1 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 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] Pirenzepine. (Perkin Elmer# NET-780)

Wash Buffer: 50 mM Hepes, pH 7.4, 500 mM 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 5-fold signal: background with ^{3}H-labeled Pirenzepine at 2 nM.

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^{o}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.

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