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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN M_3 SOMATOSTATIN RECEPTOR

CATALOG NUMBER:	HTS116M	**QUANTITY:**	200 units
LOT NUMBER:		**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). Chemicon's 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 0.72-1 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 4-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

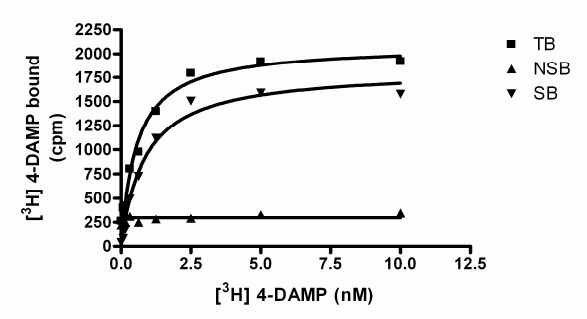

Figure 1. Saturation binding for M_3. 10 µ/well M_3 Membrane Preparation was incubated with increasing amount of [^{3}H]-4-DAMP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled atropine. Specific binding (SB) was determined by subtracting NSB from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

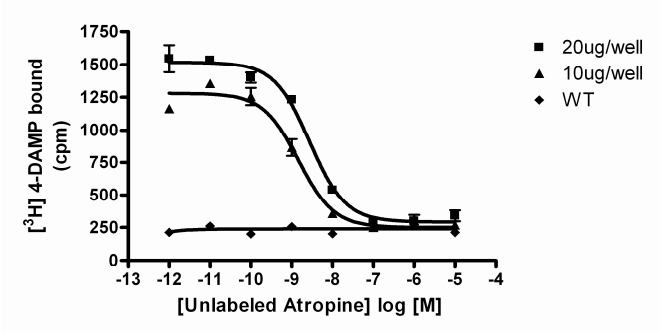

Figure 2. Competition binding for M_3. M_3 Membrane Preparation (20 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.75 nM [^{3}H]-4-DAMP and increasing concentrations of unlabeled atropine, and more than 4- fold signal:background was obtained. Sample data from a representative Lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of M_3 membrane prep.

	20 µg/well	10 µg/well
Signal:background	5.17	5.10
Specific binding (cpm)	1224	1030

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
Bmax: 3.75 pmol/mg
K_d: 0.86 nM

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

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℃

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℃

One package contains enough membranes for at least 200 assays (units), where an unit is

the amount of membrane that will yield greater than 4-fold signal:background with ^{3}H-labeled 4-DAMP at 0.75 nM

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

Packaging method: Membranes protein were 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 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Caulfield MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.

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

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION