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PRODUCT DATASHEET

ChemiScreen™ M_5 Muscarinic Acetylcholine Membrane Preparation

CATALOG NUMBER:	HTS075M	**QUANTITY:**	200 units
LOT NUMBER:	SC20180717	**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_5 receptor, along with the M_1 and M_3 receptors, signal through $G_{q/11}$ and subsequent release of Ca^{++} from the ER (Caulfield and Birdsall, 1998). Although M_5 is expressed in the CNS at relatively low levels, it appears to be the only muscarinic acetylcholine receptor expressed in midbrain dopaminergic neurons, and it has been shown to mediate dopamine release from these neurons. The M_5 receptor is also expressed in blood vessels in the brain and the periphery, and studies with M_5 knockout mice demonstrate that M_5 mediates the vasodilatory action of acetylcholine on cerebral microvessels. Further studies with M_5 knockout mice also indicate a role for M_5 in the rewarding effects of cocaine and morphine (Wess, 2004). M_5 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_5 interactions with its ligands. The membrane preparations exhibit a Kd of 0.27 nM for [^{3}H]-N-methyl-scopolamine (NMS). With 10 µg/well M_5 Membrane Prep and 1 nM [^{3}H]-NMS, greater than 3-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

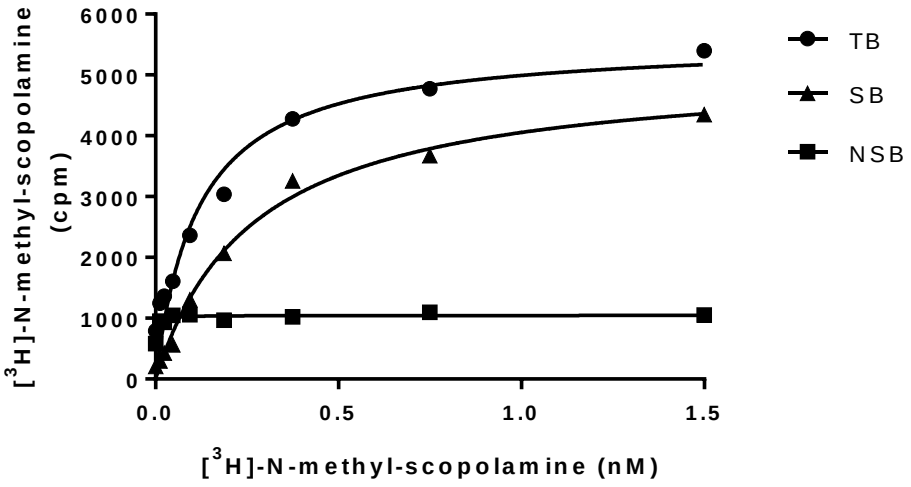

Figure 1. Saturation binding for M_5. 10 µg/well M_5 Membrane Preparation was incubated with increasing amount of [^{3}H] NMS in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Atropine. Specific binding (SB) was determined by subtracting NSB from TB.

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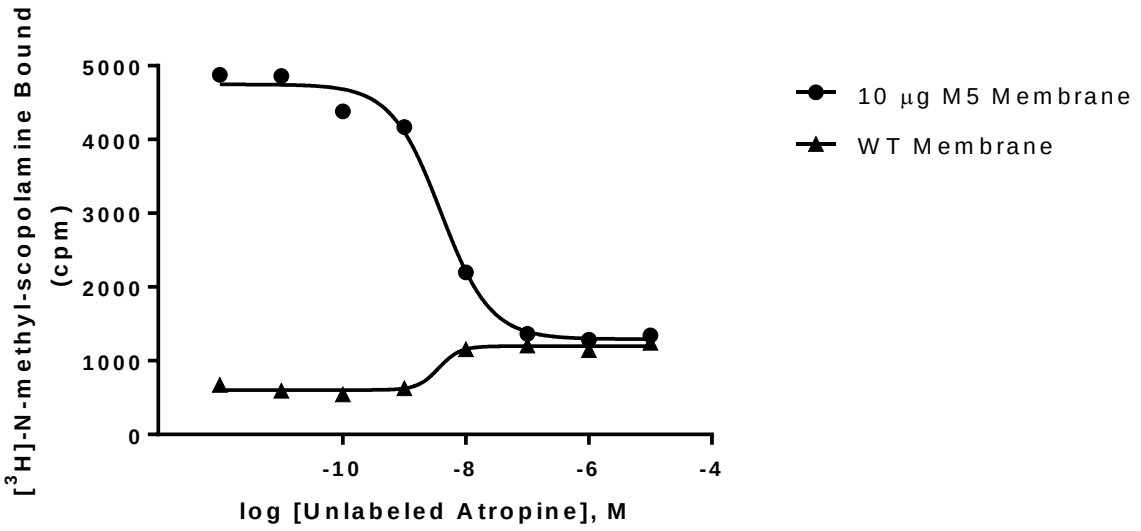

Figure 2. Competition binding for M_5. 10 µg/well M_5 Membrane Preparation (HTS075M) was incubated with 1nM [^{3}H] NMS and increasing concentrations of unlabeled Atropine. More than 3- fold signal:background ratio was obtained.

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 5.79 pmol.mg
K_d: 0.27 nM
Signal:background: >3-fold

TRANSFECTION: Full-length human CHRM5 cDNA, encoding M_5 (Accession Number: NM_012125)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous M5 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 50 mM 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] NMS (PerkinElmer#:NET636)

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 3-fold signal:background with [^{3}H] NMS at 1 nM.

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°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 M.P. and Birdsall N.J.M. (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.