

CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN M₅ MUSCARINIC ACETYLCHOLINE RECEPTOR

CATALOG NUMBER:	HTS075M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	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 M₅ receptor, along with the M₁ and M₃ receptors, signal through G_{q/11} and subsequent release of Ca⁺⁺ from the ER (Caulfield and Birdsall, 1998). Although M₅ 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 M₅ receptor is also expressed in blood vessels in the brain and the periphery, and studies with M₅ knockout mice demonstrate that M₅ mediates the vasodilatory action of acetylcholine on cerebral microvessels. Further studies with M₅ knockout mice also indicate a role for M₅ in the rewarding effects of cocaine and morphine (Wess, 2004). Millipore's M₅ 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 M₅ interactions with its ligands. The membrane preparations exhibit a K_d of 0.16 nM for [³H]-Scopolamine methyl chloride (NMS). With 10 µg/well M₅ Membrane Prep and 1 nM [³H]-NMS, greater than 5-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

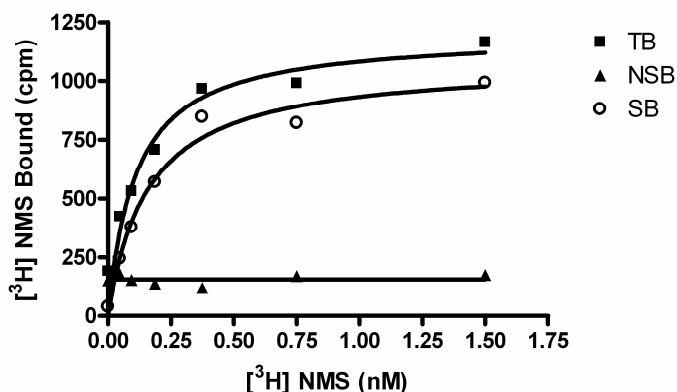


Figure 1. Saturation binding for M₅. 5 µg/well M₅ Membrane Preparation was incubated with increasing amount of ³H-labeled NMS in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Atropine. Specific binding (SB) was determined by subtracting NSB from TB.

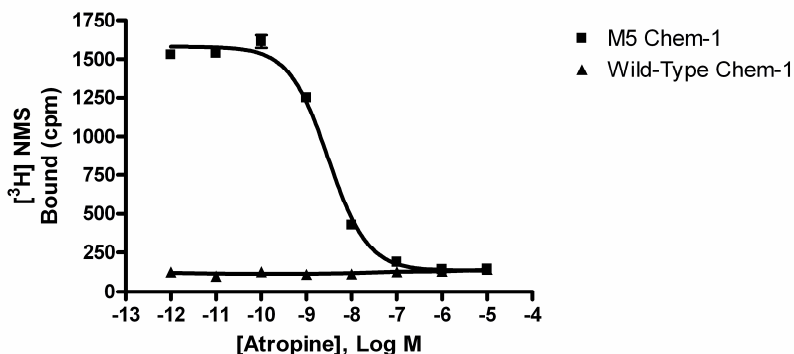


Figure 2. Competition binding for M₅. 10 µg/well M₅ Membrane Preparation (HTS075M) was incubated with 1nM ³H-labeled NMS and increasing concentrations of unlabeled Atropine. More than 5- fold signal:background ratio was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with M₅ membrane prep.

	10 µg/well
Signal:background	11.7
Specific binding (cpm)	1446

SPECIFICATIONS: 1 unit = 10 µg
 Bmax: 2.5 pmol/mg
 Kd: 0.16nM

Species: Full-length human CHRM5 cDNA, encoding M₅ (Accession Number: NM_012125)

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 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

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

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 5-fold signal:background with ³H-labeled 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: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Caulfield M.P. and Birdsall N.J.M. (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.

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