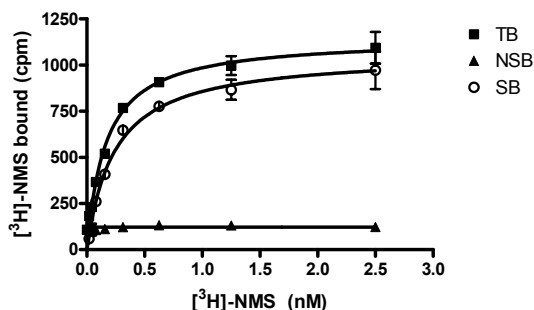


**CHEMISCREEN™ MEMBRANE PREPARATION  
RECOMBINANT HUMAN M<sub>2</sub> MUSCARINIC ACETYLCHOLINE RECEPTOR**

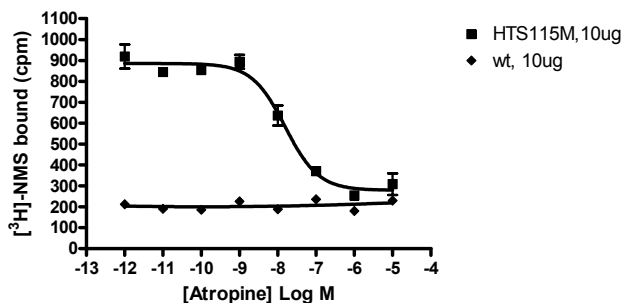
<b>CATALOG NUMBER:</b>	HTS115M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION PER VIAL:</b>	2 mL, 1 mg/mL

**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<sub>1</sub>, M<sub>3</sub> and M<sub>5</sub> receptors couple to G<sub>q</sub> to mobilize intracellular calcium, whereas the M<sub>2</sub> and M<sub>4</sub> receptors couple to G<sub>i/o</sub> to inhibit cAMP production (Caulfield and Birdsall, 1998). In urinary bladder trachea and stomach, M<sub>2</sub> augments the function of M<sub>3</sub> in promoting contractility, and activation of M<sub>2</sub> serves to counteract relaxation induced by increased cAMP levels (Ehlert et al., 2005; Wess, 2004). In addition, the ability of mAChR agonists to decrease heart rate appears to be mediated primarily by M<sub>2</sub>. Agonists of mAChRs induce tremor, hypothermia, corticosterone release, and analgesia; each of these functions is mediated at least in part by M<sub>2</sub> (Wess, 2004). Chemicon's M<sub>2</sub> 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<sub>5</sub> interactions with its ligands. The membrane preparations exhibit a K<sub>d</sub> of 0.24 nM for [<sup>3</sup>H]-Scopolamine methyl chloride (NMS). With 10 μg/well M<sub>2</sub> Membrane Prep and 0.5 nM [<sup>3</sup>H]-NMS, greater than 3-fold signal-to-background ratio was obtained.

**APPLICATIONS:** Radioligand binding assay and GTPγS binding.



**Figure 1. Saturation binding for M<sub>2</sub>.** 10 μg/well M<sub>2</sub> Membrane Preparation was incubated with increasing amount of <sup>3</sup>H-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 M<sub>2</sub>.** 10 μg/well M<sub>2</sub> Membrane Preparation (HTS115M) was incubated with 0.5 nM <sup>3</sup>H-labeled NMS and increasing concentrations of unlabeled Atropine. More than 3- fold

signal:background ratio was obtained.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with M<sub>2</sub> membrane prep and unlabeled Atropine.

	10 µg/well
Signal:background	3.2
Specific binding (cpm)	606.8

SPECIFICATIONS: 1 unit = 10 µg

B<sub>max</sub> for [<sup>3</sup>H]-NMS binding: 0.5 pmol/mg protein

K<sub>d</sub> for [<sup>3</sup>H]-NMS binding: ~0.24 nM

Species: Full-length human CHRM2 cDNA encoding M<sub>2</sub> (Accession Number: NM\_000739)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous M<sub>2</sub> 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 (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 4mM Na<sub>2</sub>HPO<sub>4</sub>, 1mM KH<sub>2</sub>PO<sub>4</sub>, pH7.4. 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 and Wash buffer: 4mM Na<sub>2</sub>HPO<sub>4</sub>, 1mM KH<sub>2</sub>PO<sub>4</sub>, pH7.4, filtered and stored at 4°C

Radioligand: [<sup>3</sup>H] NMS (Perkin Elmer#:NEX-636 )

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 <sup>3</sup>H-labeled NMS at 0.5 nM

**PRESENTATION:**

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein was 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 MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.

Ehlert FJ *et al.* (2005) The M<sub>2</sub> muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder. *J. Pharmacol. Exp. Ther.* 313: 368-378.

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

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