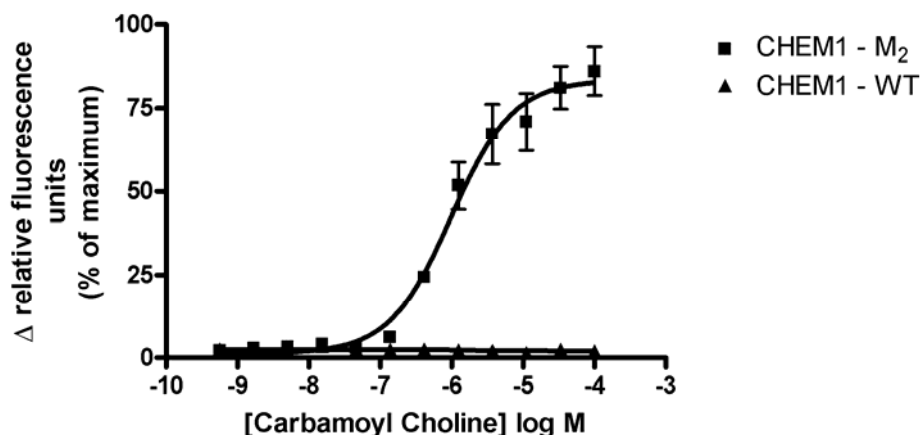


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT M<sub>2</sub> MUSCARINIC ACETYLCHOLINE RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS115C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/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 cloned human M<sub>2</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M<sub>2</sub> expression on the cell surface and contains high levels of the promiscuous G protein G<sub>α15</sub> to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between M<sub>2</sub> and its ligands.

**APPLICATIONS:** Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in M<sub>2</sub>-expressing Chem-1 cell line induced by carbamoyl choline. M<sub>2</sub>-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to carbamoyl choline (10<sup>-4</sup> to 10<sup>-9</sup> M) was determined in triplicate on a Molecular Devices FLIPR<sup>TETRA™</sup>.

**SPECIFICATIONS:** EC50 for calcium mobilization by carbamoyl choline: ~ 980 nM

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HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G $\alpha$ 15.

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

GROWTH MEDIA: DMEM containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/1x nonessential amino acids/10 mM HEPES/0.25 mg/ml Geneticin (G418)/100 U/ml each penicillin and streptomycin

**PRESENTATION:**

Cells are frozen at 2 x 10<sup>6</sup> cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

**STORAGE/HANDLING:**

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing 20 mL growth media, and place in a humidified 37°C incubator with 5% CO<sub>2</sub>. After 8-24 h, cells will adhere to the plate, at which time the media should be replaced to remove residual DMSO. Cells are passaged by washing with Ca<sup>++</sup> and Mg<sup>++</sup>-free HBSS (10 mL/T75), incubating with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5-10 minutes at 37°C, and rapping the side of the flask to dislodge the cells. Neutralize the trypsin by addition of 4 volumes growth media. Cells are typically passaged 1:10 with every 3-4 days, and should be passaged at least once after thawing prior to use in calcium flux assays.

**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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