

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT M₃ MUSCARINIC ACETYLCHOLINE RECEPTOR**

CATALOG NUMBER:	HTS116C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ 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 M₁, M₃ and M₅ receptors couple to G_q to mobilize intracellular calcium, whereas the M₂ and M₄ receptors couple to G_{i/o} to inhibit cAMP production (Caulfield and Birdsall, 1998). M₃ is expressed prominently in smooth muscle, and plays a primary role in mediating mAChR agonist-induced contractility. Mice lacking M₃ have dilated pupils, which indicates a role for M₃ in regulating tone of the pupillary sphincter muscle. In addition, M₃ plays a role in feeding, as indicated by the lean and hypophagic phenotype of M₃-null mice (Wess, 2004). Millipore's cloned human M₃-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M₃ expression on the cell surface and contains high levels of the promiscuous G protein G_{α15} to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between M₃ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

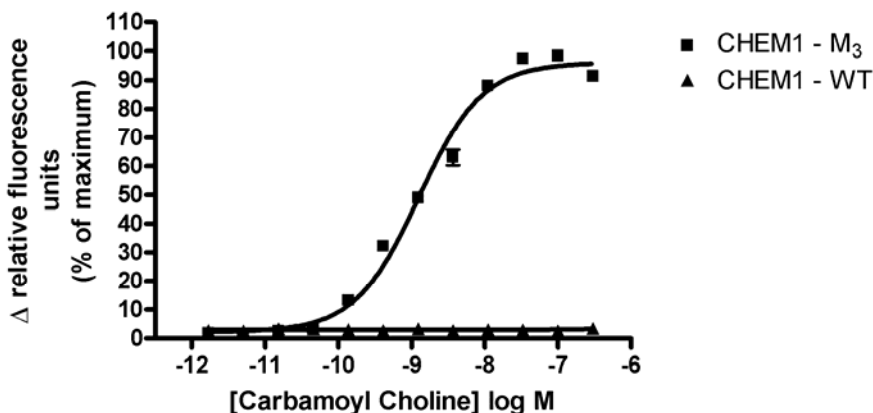


Figure 1. Calcium flux in M₃-expressing Chem-1 cell line induced by carbamoyl choline. M₃-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to carbamoyl choline (10^{-6.5} to 10⁻¹² M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

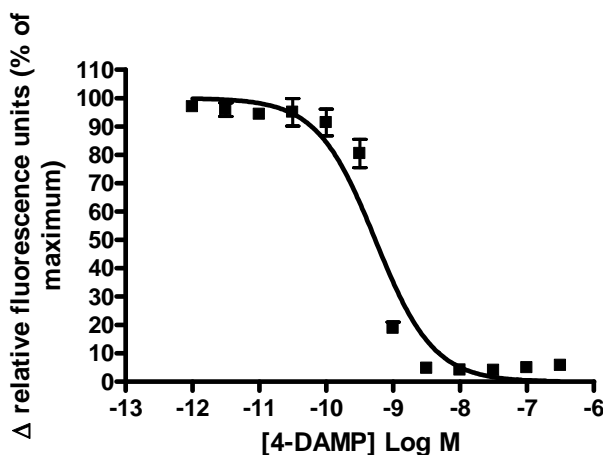


Figure 2. Assay for antagonist activity at M_3 by calcium flux assay. M_3 -expressing Chem-1 cells were loaded with Fluo-4 and washed. 4-DAMP was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to carbamoyl choline (2.4 nM) was determined in duplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC₅₀ for calcium mobilization by carbamoyl choline: ~ 1.2 nM
 IC₅₀ for 4-DAMP: ~0.54 nM
 Z' = 0.82 with carbamoyl choline at 2x EC₅₀

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

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

PRESENTATION:

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

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. 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 growth media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca⁺⁺ and Mg⁺⁺ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO₂ until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.

5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5×10^6 cells/mL in Chem-1 Freezing Media (cell densities of $2-10 \times 10^6$ are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

MEDIA:

Chem-1 Growth Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250 μg /mL Genetecin/G-418

Chem-1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

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.

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HUMAN RECOMBINANT M₃ MUSCARINIC ACETYLCHOLINE RECEPTOR****Product No. HTS116C**

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