

PRODUCT DATASHEET

ChemiScreen™ M₄ Muscarinic Acetylcholine Receptor Stable Cell Line

CATALOG NUMBER: HTS117C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N₂.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-4 host, which supports high levels of functional receptor expression on the cell surface. Chem-4 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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). Muscarinic M₄ receptor is known to be abundantly expressed in the striatum (Levey 1993). Consistent with this fact, locomotor activity was significantly increased in M₄ receptor-deficient mice (Gomez *et al.* 1999). The cloned human M₄-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant M₄ expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between M₄ and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

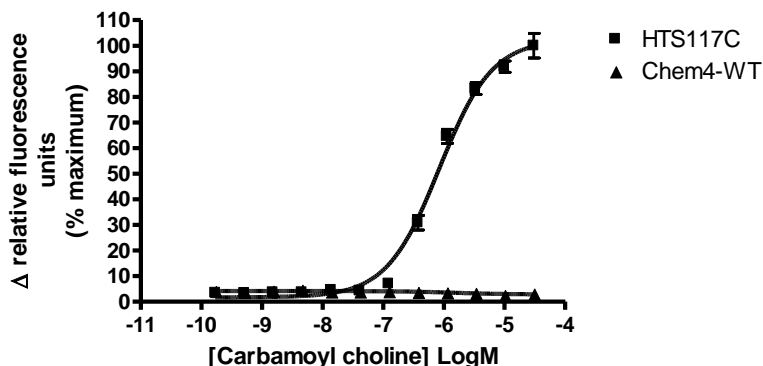


Figure 1. Representative data for activation of the M₄ receptor stably expressed in Chem-4 cells induced by Acetylcholine using a fluorescent calcium flux assay. M₄-expressing Chem-4 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-4) were tested to determine the specificity of the resulting signal.

Table 1. EC₅₀ value of M₄-expressing Chem-4 cells.

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
Acetylcholine	Calcium Flux - Fluorescence	840	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC₅₀ and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Hygromycin	250 µg/ml	Invivogen: ant-hg-5
	Sterile PBS	-	Hyclone: SH30028.03
CryoMedium	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37°C, 5% CO₂.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%. At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37° C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm ²)	Volume (mL)	Total Cell Number (x10 ⁶)	Growth Period (hrs)
T75	15	5.0	24
T75	15	3.0	48
T75	15	0.5	72

ASSAY SETUP

Fluorescence

Table 4. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Acetylcholine ligand	Sigma: A6625
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-4, an adherent cell line expressing the promiscuous G-protein, G α 15.

EXOGENOUS GENE EXPRESSION

Human M₄ cDNA (Accession Number: NM_00741) and promiscuous G protein are expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTSCHEM-4	ChemiScreen™ Chem-4 Parental Cell Line (control cells)
HTS117M	ChemiScreen™ M ₄ Muscarinic Acetylcholine Receptor Membrane Prep

REFERENCES

1. Caulfield MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.
2. Levey AI (1993). Immunological localization of M1-M5 muscarinic acetylcholine receptors in peripheral tissue and brain. *Life Sci.* 52: 441-448.
3. Gomeza J *et al.* (1999) Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. USA* 96: 10483-10488.

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