

PRODUCT DATASHEET

ChemiScreen™ M₁ Acetylcholine (Muscarinic) Receptor Stable Cell Line

CATALOG NUMBER: HTS044C

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-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 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 family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The M₁ receptor, along with the M₃ and M₅ receptors, signal through G_{q/11} and subsequent release of Ca⁺⁺ from the ER. The M₁ receptor is expressed in ganglia and mediates depolarization of ganglia by inhibition of voltage-gated M-type K⁺ channels. In addition, the M₁ receptor mediates venous contraction (Caulfield and Birdsall, 1998). Cloned human M₁ receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with M₁. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the M₁ receptor.

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

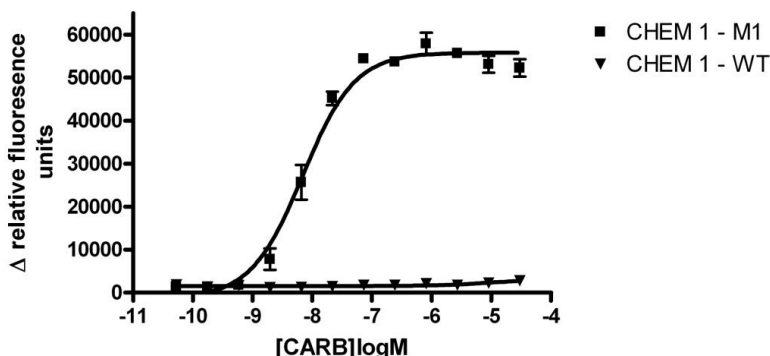


Figure 1. Representative data for activation of M₁ receptor stably expressed in Chem-1 cells induced by Carbamoylcholine using a fluorescent calcium flux assay. M₁-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 7,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC₅₀ value of M₁-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
Carbamoylcholine	Calcium Flux - Fluorescence	100	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	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	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	2.0	48
T75	15	0.45	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 TM , AM	AAT Bioquest: 21080
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, $5 \times 10^5 / \text{ml} = 10 \text{ mL volume}$)
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-1, an adherent cell line expressing the promiscuous G-protein, G α 15. [OR other (e.g., CHO-K1, 1321N1, U2OS)]

EXOGENOUS GENE EXPRESSION

CHRM1 cDNA (Accession Number: NM_000738; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

Nucleotide:

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ATG AAC ACT TCA GCC CCA CCT GCT GTC ACA AAC ATC ACC GTC CTG GCA CCA GGA AAG GGT CCC TGG CAA GTG GCC TTC ATT
GGG ATC ACC ACC GGC CTC CTG TCG GTA GCC ACA GTG ACA GGC AAC CTG CTG GTA CTC ATC TCT TTC AAG GTC AAC ACG GAG CTC
AAG ACA GTC AAT AAC TAC TTC CTG CTG AGC CTG GCC TGT GCT GAC CTC ATC ATC GGT ACC TTC TCC ATG AAC CTC TAT ACC ACG
TAC CTG CTC ATG GGC CAC TGG GCT CTG GGC ACG CTG GCT TGT GAC CTC TGG CTG GCC CTG GAC TAT GTG GCC AGC AAT GCC TCC
GTC ATG AAT CTG CTG CTC ATC AGC TTT GAC CGC TAC TTC TCC GTG ACT CGG CCC CTG AGC TAC CGT GCC AAG CGC ACA CCC CGC
CGG GCA GCT CTG ATG ATC GGC CTG GCC TGG CTG GTT TCC TTT GTG CTC TGG GCC CCA GCC ATC CTC TTC TGG CAG TAC CTG GTA
GGG GAG CGG ACA GTG CTA GCT GGG CAG TGC TAC ATC CAG TTC CTC TCC CAG CCC ATC ATC ACC TTT GGC ACA GCC ATG GCT GCC
TTC TAC CTC CCT GTC ACA GTC ATG TGC ACG CTC TAC TGG CGC ATC TAC CGG GAG ACA GAG AAC CGA GCA CGG GAG CTG GCA GCC
CTT CAG GGC TCC GAG ACG CCA GGC AAA GGG GGT GGC AGC AGC AGC AGC TCA GAG AGG TCT CAG CCA GGG GCT GAG GGC TCA CCA
GAG ACT CCT CCA GGC CGC TGC TGT CGC TGC TGC CGG GCC CCC AGG CTG CTG CAG GCC TAC AGC TGG AAG GAA GAA GAG GAA GAG
GAC GAA GGC TCC ATG GAG TCC CTC ACA TCC TCA GAG GGA GAG GAG CCT GGC TCC GAA GTG GTG ATC AAG ATG CCA ATG GTG GAC
CCC GAG GCA CAG GCC CCC ACC AAG CAG CCC CCA CGG AGC TCC CCA AAT ACA GTC AAG AGG CCG ACT AAG AAA GGG CGT GAT CGA
GCT GGC AAG GGC CAG AAG CCC CGT GGA AAG GAG CAG CTG GCC AAG CGG AAG ACC TTC TCG CTG GTC AAG GAG AAG AAG GCG GCT
CGG ACC CTG AGT GCC ATC CTC CTG GCC TTC ATC CTC AC CCG TAC AAC ATC ATG GTG CTG GTG TCC ACC TTC TGC AAG
GAC TGT GTT CCC GAG ACC CTG TGG GAG CTG GGC TAC TGG CTG TGC TAC GTC AAC AGC ACC ATC AAC CCC ATG TGC TAC GCA CTC
TGC AAC AAA GCC TTC CGG GAC ACC TTT CGC CTG CTG CTG CTT TGC CGC TGG GAC AAG AGA CGC TGG CGC AAG ATC CCC AAG CGC
CCT GGC TCC GTG CAC CGC ACT CCC TCC CGC CAA TGC TGA
    
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Amino Acid:

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MNTSAPPVAVSPNITVLPAGKGPWQVAFIGITTTGLLSLATVVTGNLLVLI SFKVNTLKTVN
NYFLLSLACADLIIGTFSMNLTYTTYLLMGHWALGTLACDLWLALDYVNASVSMNLLIS
FDRYFSVTRPLSYRAKRTPRRAALMIGLAWLVSVFLWAPAILFWQYLVGERTVLAGQCYI
QFLSQPIITFGTAMAAFYLPVTVMCTLYWRIYRETNRARELAALQGSSETPGKGGSSSS
SERSQPGAEGSPETPPGRCRCRPRLLQAYSWKEEEEDEGSMESLTSSEGEFPGSEV
VTKMFMVDPEAQAPTQPPRSPNTVKRPTKKGRDRAGKQKPRGKEQLAKRKTFSLVKE
KKAARTLSAILLAFILTWTPYNIIMVLVSTFCKDCVPEITLWELGYWLCYVNSTINPMCYAL
CNKAFRDTRFLLLLCRWDKRRWRKI PKRPGSVHRTFSRQC*
    
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS044M	ChemiScreen™ M1 acetylcholine (muscarinic) receptor membrane prep

REFERENCES

1. Caulfield M.P. and Birdsall N.J.M. (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50: 279-290.

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