eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ M_5 Muscarinic Acetylcholine Receptor Stable Cell Line

CATALOG NUMBER: HTS075C

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

STORAGE: Vials are to be stored in liquid N_2.

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 M_5 receptor, along with the M_1 and M_3 receptors, signal through $G_{q/11}$ and subsequent release of Ca^{++} from the ER (Caulfield and Birdsall, 1998). Although M_5 is expressed in the CNS at relatively low levels, it appears to be the only muscarinic acetylcholine receptor expressed in midbrain dopaminergic neurons, and it has been shown to mediate dopamine release from these neurons. The M_5 receptor is also expressed in blood vessels in the brain and the periphery, and studies with M_5 knockout mice demonstrate that M_5 mediates the vasodilatory action of acetylcholine on cerebral microvessels. Further studies with M_5 knockout mice also indicate a role for M_5 in the rewarding effects of cocaine and morphine (Wess, 2004). The cloned human M_5-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M_5 expression on the cell surface and contains high levels of the promiscuous G protein $G_{\alpha 15}$ to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between M_5 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.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

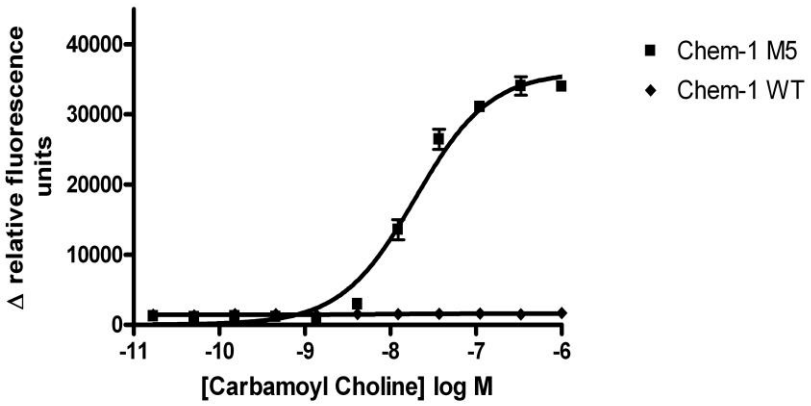

Figure 1. Representative data for activation of the M_5 receptor stably expressed in Chem-1 cells induced by Acetylcholine using a fluorescent calcium flux assay. M_5–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a 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 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of M_5-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Acetylcholine	Calcium Flux - Fluorescence	19.5	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} 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 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC 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, 37ºC, 5% CO_2.
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 37º 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	4.5	24
T75	15	2.0	48
T75	15	0.35	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 $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=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 37ºC 5% CO_2 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, Gα15.

EXOGENOUS GENE EXPRESSION

Human M_5 cDNA (Accession Number: NM_012125; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                ATG GAA GGG GAT TCT TAC CAC AAT GCA ACC ACC GTC AGT GGC ACC

                                                 M   E   G   D   S   Y   H   N   A   T   T   V   S   G   T

CCA GTA AAT CAC CAG CCT TTG GAA CGC CAC AGG TTG TGG GAA GTC ATC ACC ATT GCA GCT GTG ACT GCT

 P   V   N   H   Q   P   L   E   R   H   R   L   W   E   V   I   T   I   A   A   V   T   A
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GTG GTA AGC CTG ATC ACC ATT GTG GGC AAT GTC TTG GTC ATG ATC TCC TTC AAA GTC AAC AGC CAG CTC
 V   V   S   L   I   T   I   V   G   N   V   L   V   M   I   S   F   K   V   N   S   Q   L
AAG ACA GTT AAC AAC TAT TAC CTG CTC AGC TTA GCC TGT GCA GAT CTC ATC ATT GGA ATC TTC TCC ATG
 K   T   V   N   N   Y   Y   L   L   S   L   A   C   A   D   L   I   I   G   I   F   S   M
AAC CTC TAC ACC ACC TAC ATC CTC ATG GGA CGC TGG GCT CTC GGG AGT CTG GCT TGT GAC CTT TGG CTT
 N   L   Y   T   T   Y   I   L   M   G   R   W   A   L   G   S   L   A   C   D   L   W   L
GCA CTG GAC TAC GTG GCC AGC AAC GCT TCT GTC ATG AAC CTT CTG GTG ATC AGT TTT GAC CGT TAC TTT
 A   L   D   Y   V   A   S   N   A   S   V   M   N   L   L   V   I   S   F   D   R   Y   F
TCC ATC ACA AGA CCC TTG ACA TAT CGG GCC AAG CGT ACT CCG AAA AGG GCT GGC ATC ATG ATT GGC TTG
 S   I   T   R   P   L   T   Y   R   A   K   R   T   P   K   R   A   G   I   M   I   G   L
GCC TGG CTG ATC TCC TTC ATC CTC TGG GCC CCA GCA ATC CTC TGC TGG CAG TAC TTG GTT GGG AAG CGG
 A   W   L   I   S   F   I   L   W   A   P   A   I   L   C   W   Q   Y   L   V   G   K   R
ACA GTT CCA CTG GAT GAG TGC CAG ATC CAG TTT CTC TCT GAG CCC ACC ATC ACT TTT GGC ACT GCC ATT
 T   V   P   L   D   E   C   Q   I   Q   F   L   S   E   P   T   I   T   F   G   T   A   I
GCT GCC TTC TAC ATC CCT GTT TCT GTC ATG ACC ATC CTC TAC TGT CGA ATC TAC CGG GAA ACA GAG AAG
 A   A   F   Y   I   P   V   S   V   M   T   I   L   Y   C   R   I   Y   R   E   T   E   K
CGA ACC AAG GAC CTG GCT GAC CTC CAG GGT TCT GAC TCT GTG ACC AAA GCT GAG AAG AGA AAG CCA GCT
 R   T   K   D   L   A   D   L   Q   G   S   D   S   V   T   K   A   E   K   R   K   P   A
CAT AGG GCT CTG TTC AGA TCC TGC TTG CGC TGT CCT CGA CCC ACC CTG GCT CAG CGG GAA AGG AAC CAG
 H   R   A   L   F   R   S   C   L   R   C   P   R   P   T   L   A   R   Q   E   R   N   Q
GCC TCC TGG TCA TCC TCC CGC AGG AGC ACC TCC ACC ACT GGG AAG CCA TCC CAA GCC ACT GGC CCA AGC
 A   S   W   S   S   S   R   R   S   T   S   T   T   G   K   P   S   Q   A   T   G   P   S
GCC AAT TGG GCC AAA GCT GAG CAG CTC ACC ACC TGT AGC AGC TAC CCT TCC TCA GAG GAT GAG GAC AAG
 A   N   W   A   K   A   E   Q   L   T   T   C   S   S   Y   P   S   S   E   D   E   D   K
CCC GCC ACT GAC CCT GTC CTC CAA GTG GTC TAC AAG AGT CAG GGT AAG GAA AGC CCA GGG GAA GAA TTC
 P   A   T   D   P   V   L   Q   V   V   Y   K   S   Q   G   K   E   S   P   G   E   E   F
AGT GCT GAA GAG ACT GAG GAA ATT TTT GTG AAA GCT GAA ACT GAA AAA AGT GAC TAT GAC ACC CCA AAC
 S   A   E   E   T   E   E   I   F   V   K   A   E   T   E   K   S   D   Y   D   T   P   N
TAC CTT CTG TCT CCA GCA GCT GCT CAT AGA CCC AAG AGT CAG AAA TGT GTG GCC TAT AAG TTC CGA TTG
 Y   L   L   S   P   A   A   A   H   R   P   K   S   Q   K   C   V   A   Y   K   F   R   L
GTG GTA AAA GCT GAC GGG AAC CAG GAG ACC AAC AAT GGC TGT CAC AAG GTG AAA ATC ATG CCC TGC CCC
 V   V   K   A   D   G   N   Q   E   T   N   N   G   C   H   K   V   K   I   M   P   C   P
TTC CCA GTG GCC AAG GAA CCT TCA ACG AAA GGC CTC AAT CCC AAC CCC AGC CAT CAA ATG ACC AAA CGA
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 F   P   V   A   K   E   P   S   T   K   G   L   N   P   N   P   S   H   Q   M   T   K   R

AAG AGA GTG GTC CTA GTC AAA GAG AGG AAA GCA GCC CAG ACA CTG AGT GCC ATT CTC CTG GCC TTC ATC

 K   R   V   V   L   V   K   E   R   K   A   A   Q   T   L   S   A   I   L   L   A   F   I

ATC ACA TGG ACC CCG TAT AAC ATC ATG GTC CTG GTT TCT ACC TTC TGT GAC AAG TGT GTC CCA GTC ACC

 I   T   W   T   P   Y   N   I   M   V   L   V   S   T   F   C   D   K   C   V   P   V   T

CTG TGG CAC TTG GGC TAT TGG TTG TGC TAT GTC AAT AGC ACT GTC AAC CCC ATC TGC TAT GCC CTC TGC

 L   W   H   L   G   Y   W   L   C   Y   V   N   S   T   V   N   P   I   C   Y   A   L   C

AAC AGA ACC TTC AGG AAG ACC TTT AAG ATG CTG CTT CTC TGC CGA TGG AAA AAG AAA AAA GTG GAA GAG

 N   R   T   F   R   K   T   F   K   M   L   L   L   C   R   W   K   K   K   K   V   E   E

AAG TTG TAC TGG CAG GGG AAC AGC AAG CTA CCC TGA

 K   L   Y   W   Q   G   N   S   K   L   P  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS075M	ChemiScreen™ M_5 Muscarinic Acetylcholine 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.
2. Wess J. (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.

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