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PRODUCT DATASHEET

ChemiScreen™ μ (Mu) Opioid Receptor Stable Cell Line

CATALOG NUMBER: HTS101C

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-5 host, which supports high levels of functional receptor expression on the cell surface. Chem-5 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Opiates derived from the opium poppy, *Papaver somniferum*, have been used in for millenia for their anti-diarrheal, analgesic and euphoric properties. More recently, endogenous peptides, enkephalins, dynorphins, and endorphins, were found to bind to the same sites as opiate alkaloids. The receptors for the classical opioids are three related GPCRs, μ, κ, and δ, that activate Gi/o to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor (Dhawan *et al.*, 1996). The cloned human μ-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant μ expression on the cell surface and contains high levels of the promiscuous G protein 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 μ 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

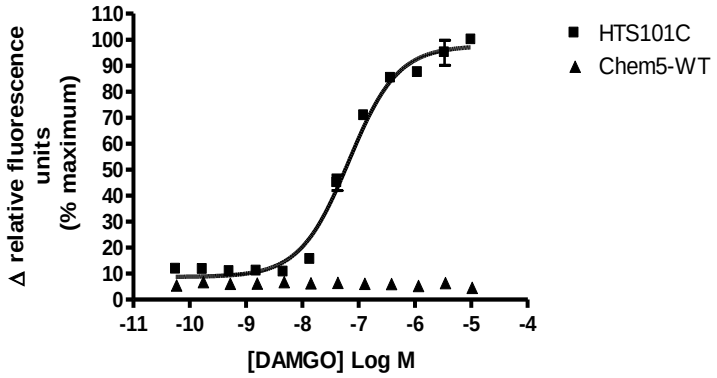

Figure 1. Representative data for activation of the μ receptor stably expressed in Chem-5 cells induced by DAMGO using a fluorescent calcium flux assay. μ–expressing Chem-5 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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-5) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of μ-expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
DAMGO	Calcium Flux - Fluorescence	67	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
	Hygromycin	500 µg/ml	Invivogen: ant-hg-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	5.0	24
T75	15	2.0	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
DAMGO ligand	Sigma: E7384
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.

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HOST CELL

Chem-5, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human μ cDNA (Accession Number: NM_000914; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                                ATG TCA GAT GCT CAG CTC GGT CCC CTC CGC

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

Product Number	Description
HTSCHEM-5	ChemiScreen™ Chem-5 Parental Cell Line (control cells)
HTS101M	ChemiScreen™ μ (Mu) Opioid Receptor Membrane Prep

REFERENCES

1. Dhawan BN *et al.* (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48: 567-92.

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