

## PRODUCT DATASHEET

### ChemiScreen™ $\delta$ Opioid Receptor Stable Cell Line

#### CATALOG NUMBER: HTS100C

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

**STORAGE:** Vials are to be stored in liquid N<sub>2</sub>.

#### 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 G $\alpha$ 15, 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,  $\delta$ ,  $\kappa$ , and  $\mu$ , (also known as OP<sub>1</sub>, OP<sub>2</sub> and OP<sub>3</sub>, respectively), that activate G<sub>i/o</sub> to reduce intracellular cAMP levels. Although most clinically used opioids function by activation of the  $\mu$  opioid receptor, agonists of spinal  $\delta$  opioid receptors have antinociceptive activity that is independent of  $\mu$ . In addition, activation of  $\delta$  increases locomotor activity, inhibits gastrointestinal motility, and decreases respiratory frequency (Dhawan *et al.*, 1996). Agonists for  $\delta$  opioid receptors also exhibit antidepressant-like activity in animal models (Broom *et al.*, 2002). The cloned human  $\delta$  receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with  $\delta$ . These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the  $\delta$  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

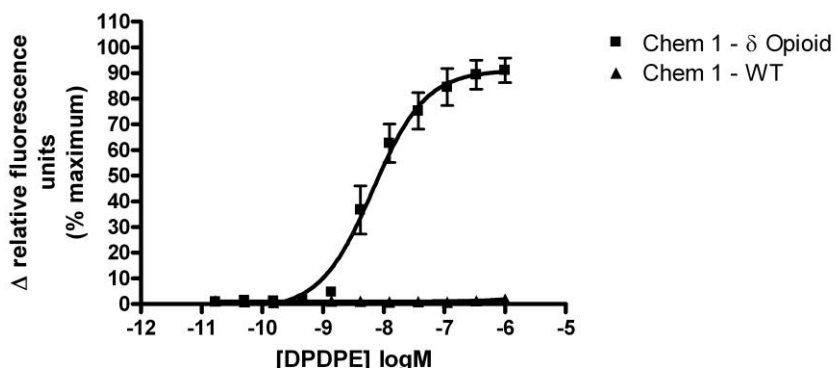


Figure 1. Representative data for activation of  $\delta$  receptor stably expressed in Chem-1 cells induced by DPDPE using a fluorescent calcium flux assay.  $\delta$ -expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with 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<sup>TETRA</sup>® 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. EC<sub>50</sub> value of  $\delta$  -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC <sub>50</sub> (nM)	REFERENCE
DPDPE	Calcium Flux - Fluorescence	6.6	Eurofins Internal Data

\* The cell line was tested and found to have equivalent EC<sub>50</sub> 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% CO<sub>2</sub>.
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 <sup>2</sup> )	Volume (mL)	Total Cell Number (x10 <sup>6</sup> )	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<sup>TETRA</sup>® 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
DPDPE ligand	Tocris: 1431
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  $5 \times 10^5$  cells/ml (i.e, if collected  $5 \times 10^6$  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% CO<sub>2</sub> 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<sup>TETRA</sup>® 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

OPRD1 cDNA (Accession Number: NM\_000911; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary PHS plasmid.

**CODING SEQUENCE**

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

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**RELATED PRODUCTS**
**Product Number**
**Description**

**HTSCHEM-1** ChemiScreen™ Chem-1 Parental Cell Line (control cells)

**HTS100M** ChemiScreen™ Delta Opioid receptor membrane prep

**REFERENCES**

1. Broom DC *et al.* (2002) Behavioral effect of  $\delta$ -opioid receptor agonists: potential antidepressants? *Jpn. J. Pharmacol.* 90: 1-6.
2. Dhawan BN *et al.* (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48: 567-92:

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