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# PRODUCT DATASHEET

## ChemiScreen™ κ Opioid Receptor Stable Cell Line

## CATALOG NUMBER: HTS095C

**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.

Opiates derived from the opium poppy, *Papaver somniferum*, have been used 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 $G_{i/o}$ to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor. Activation of the κ opioid receptor by selective agonists also produces analgesia, primarily mediated by spinal sites, but causes dysphoria and psychosis instead of euphoria. The κ receptor at central and peripheral sites is also largely responsible for the anti-diarrheal effects of opiates (Dhawan *et al.*, 1996). The cloned human κ receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with κ and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the κ receptor.

## USE RESTRICTIONS

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## 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

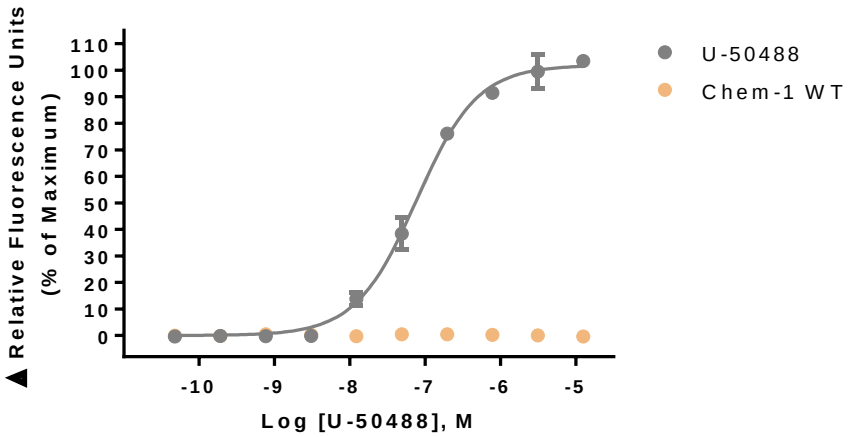


Figure 1. Representative data for activation of κ receptor stably expressed in Chem-1 cells induced by U-50488 using a fluorescent calcium flux assay. κ–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium 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 2,100 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

| LIGAND | ASSAY | POTENCY $EC_{50}$ (nM) | REFERENCE |
|---|---|---|---|
| **U-50488** | Calcium Flux - Fluorescence | 76 | 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. The Z' value, as defined with response to 10µM U-50488, was 0.7.

## 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 | Hyclone: SH30237.01 |
| **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 | 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™, AM | AAT Bioquest: 21080 |
| U-50488 ligand | Sigma: D8040 |
| 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-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

## EXOGENOUS GENE EXPRESSION

Human OPRK1 cDNA (Accession Number: NM_000912; see CODING SEQUENCE below)

## CODING SEQUENCE

```
                                                ATG GAC TCC CCG ATC CAG ATC TTC CGC GGG GAG CCT
                                                  M   D   S   P   I   Q   I   F   R   G   E   P

GGC CCT ACC TGC GCC CCG AGC GCC TGC CTG CCC CCC AAC AGC AGC GCC TGG TTT CCC GGC TGG GCC GAG
 G   P   T   C   A   P   S   A   C   L   P   P   N   S   S   A   W   F   P   G   W   A   E

CCC GAC AGC AAC GGC AGC GCC GGC TCG GAG GAC GCG CAG CTG GAG CCC GCG CAC ATC TCC CCG GCC ATC
 P   D   S   N   G   S   A   G   S   E   D   A   Q   L   E   P   A   H   I   S   P   A   I

CCG GTC ATC ATC ACG GCG GTC TAC TCC GTA GTG TTC GTC GTG GGC TTG GTG GGC AAC TCG CTG GTC ATG
 P   V   I   I   T   A   V   Y   S   V   V   F   V   V   G   L   V   G   N   S   L   V   M

TTC GTG ATC ATC CGA TAC ACA AAG ATG AAG ACA GCA ACC AAC ATT TAC ATA TTT AAC CTG GCT TTG GCA
 F   V   I   I   R   Y   T   K   M   K   T   A   T   N   I   Y   I   F   N   L   A   L   A

GAT GCT TTA GTT ACT ACA ACC ATG CCC TTT CAG AGT ACG GTC TAC TTG ATG AAT TCC TGG CCT TTT GGG
 D   A   L   V   T   T   T   M   P   F   Q   S   T   V   Y   L   M   N   S   W   P   F   G

GAT GTG CTG TGC AAG ATA GTA ATT TCC ATT GAT TAC TAC AAC ATG TTC ACC AGC ATC TTC ACC TTG ACC
 D   V   L   C   K   I   V   I   S   I   D   Y   Y   N   M   F   T   S   I   F   T   L   T

ATG ATG AGT GTG GAC CGC TAC ATT GCC GTG TGC CAC CCC GTG AAG GCT TTG GAC TTC CGC ACA CCC TTG
 M   M   S   V   D   R   Y   I   A   V   C   H   P   V   K   A   L   D   F   R   T   P   L

AAG GCA AAG ATC ATC AAT ATC TGC ATC TGG CTG CTG TCG TCA TCT GTT GGC ATC TCT GCA ATA GTC CTT
 K   A   K   I   I   N   I   C   I   W   L   L   S   S   S   V   G   I   S   A   I   V   L

GGA GGC ACC AAA GTC AGG GAA GAC GTC GAT GTC ATT GAG TGC TCC TTG CAG TTC CCA GAT GAT GAC TAC
 G   G   T   K   V   R   E   D   V   D   V   I   E   C   S   L   Q   F   P   D   D   D   Y

TCC TGG TGG GAC CTC TTC ATG AAG ATC TGC GTC TTC ATC TTT GCC TTC GTG ATC CCT GTC CTC ATC ATC
 S   W   W   D   L   F   M   K   I   C   V   F   I   F   A   F   V   I   P   V   L   I   I

ATC GTC TGC TAC ACC CTG ATG ATC CTG CGT CTC AAG AGC GTC CGG CTC CTT TCT GGC TCC CGA GAG AAA
 I   V   C   Y   T   L   M   I   L   R   L   K   S   V   R   L   L   S   G   S   R   E   K

GAT CGC AAC CTG CGT AGG ATC ACC AGA CTG GTC CTG GTG GTG GTG GCG GTC TTC GTC GTC TGC TGG ACT
 D   R   N   L   R   R   I   T   R   L   V   L   V   V   V   A   V   F   V   V   C   W   T

CCC ATT CAC ATA TTC ATC CTG GTG GAG GCT CTG GGG AGC ACC TCC CAC AGC ACA GCT GCT CTC TCC AGC
 P   I   H   I   F   I   L   V   E   A   L   G   S   T   S   H   S   T   A   A   L   S   S

TAT TAC TTC TGC ATC GCC TTA GGC TAT ACC AAC AGT AGC CTG AAT CCC ATT CTC TAC GCC TTT CTT GAT
 Y   Y   F   C   I   A   L   G   Y   T   N   S   S   L   N   P   I   L   Y   A   F   L   D

GAA AAC TTC AAG CGG TGT TTC CGG GAC TTC TGC TTT CCA CTG AAG ATG AGG ATG GAG CGG CAG AGC ACT
 E   N   F   K   R   C   F   R   D   F   C   F   P   L   K   M   R   M   E   R   Q   S   T

AGC AGA GTC CGA AAT ACA GTT CAG GAT CCT GCT TAC CTG AGG GAC ATC GAT GGG ATG AAT AAA CCA GTA
 S   R   V   R   N   T   V   Q   D   P   A   Y   L   R   D   I   D   G   M   N   K   P   V

TGA
Stp
```

## RELATED PRODUCTS

| Product Number | Description |
|---|---|
| **HTSCHEM-1** | ChemiScreen™ Chem-1 Parental Cell Line (control cells) |
| **HTS095M** | ChemiScreen™ κ Opioid family 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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