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PRODUCT DATASHEET

Ready-to-Assay™ delta Opioid Receptor Frozen Cells

CATALOG NUMBER: HTS100RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

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, δ, κ, and μ, (also known as OP_1, OP_2 and OP_3, respectively), that activate $G_{i/o}$ to reduce intracellular cAMP levels. Although most clinically used opioids function by activation of the μ opioid receptor, agonist of spinal δ opioid receptors have antinociceptive activity that is independent of μ. In addition, activation of δ increases locomotor activity, inhibits gastrointestinal motility, and decreases respiratory frequency (Dhawan *et al.*, 1996). Agonists for δ opioid receptors also exhibit antidepressant-like activity in animal models (Broom *et al.*, 2002). Cloned human δ opioid - expressing cell line is made in the Chem-1 host, which supports high levels of recombinant δ opioid expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at δ.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

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.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

eurofins | Discovery Services

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

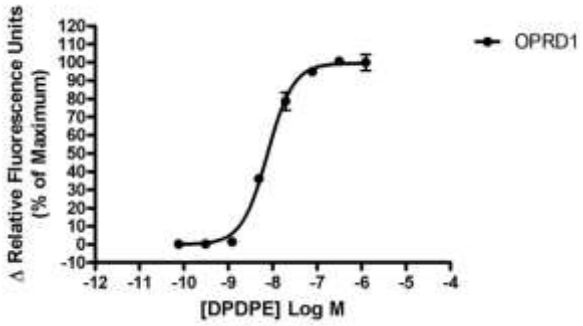

Figure 1. Representative data for activation of δ opioid receptor. Calcium flux in δ opioid –expressing Chem-1 cell line induced by DPDPE. δ opioid –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
DPDPE	Calcium Flux	8	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).

11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
DPDPE ligand	Tocris: 1431
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

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	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS 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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS100M	ChemiScreen™ Delta Opioid receptor membrane prep

REFERENCES

1. Broom DC *et al.* (2002) Behavioral effect of ▪-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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