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PRODUCT DATASHEET

Ready-to-Assay™ Kappa Opioid Receptor Frozen Cells

CATALOG NUMBER: HTS095RTA
Lot: 22F0701

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 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). Cloned human κ-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant κ expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway.

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.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

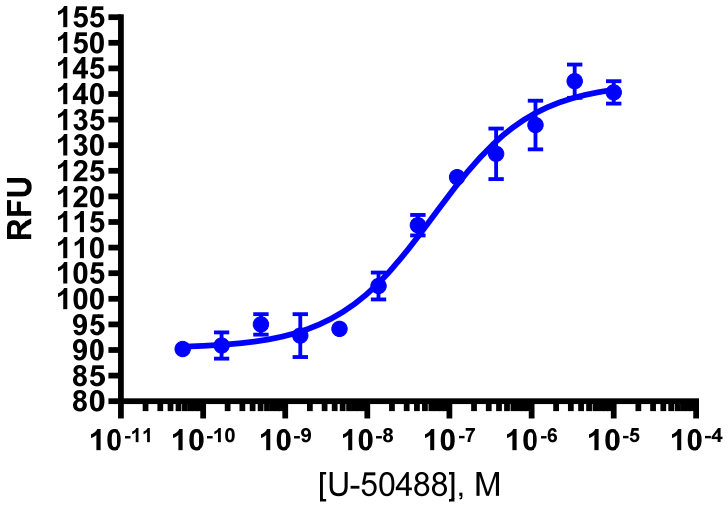

Figure 1. Representative data for activation of κ receptor. Calcium flux in κ-expressing Chem-1 cell line induced by U-50488. κ-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 3-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}.

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
U-50488	Calcium Flux	64	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% CO_2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and remove 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
U-50488 ligand	Sigma: D8040
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

Full-length human OPRK1 cDNA (Accession Number: NM_000912; see CODING SEQUENCE below) encoding the κ opioid receptor.

CODING SEQUENCE

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                                                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
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS095M	ChemiScreen™ Kappa Opioid Receptor Membrane Prep

HTS100RTA	Ready-to-Assay™ Delta Opioid Receptor Frozen Cells
HTS100M	ChemiScreen™ Delta Opioid Receptor Membrane Prep
HTS101RTA	Ready-to-Assay™ Mu Opioid Receptor Frozen Cells
HTS101M	ChemiScreen™ 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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