eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ FP Receptor Frozen Cells

CATALOG NUMBER: HTS093RTA

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.

Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and subsequently by isomerases and synthases. Cells rapidly secrete prostanoids after synthesis, whereupon the prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostaglandin $PGF_{2\alpha}$ binds specifically to the FP receptor, which couples to $G_{q/11}$ to mobilize intracellular calcium. Binding of $PGF_{2\alpha}$ to FP receptors in the corpus luteum is required for luteolysis and subsequent parturition in mice (Sugimoto *et al.*, 1998). $PGF_{2\alpha}$ also decreases intraocular pressure by an FP-dependent mechanism, and an $PGF_{2\alpha}$ analog, latanoprost, is used clinically in the treatment of glaucoma (Crowston *et al.*, 2004). FP also contributes to tachycardia induced by inflammatory stimuli (Takayama *et al.*, 2005). FP-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant FP expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at FP.

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

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

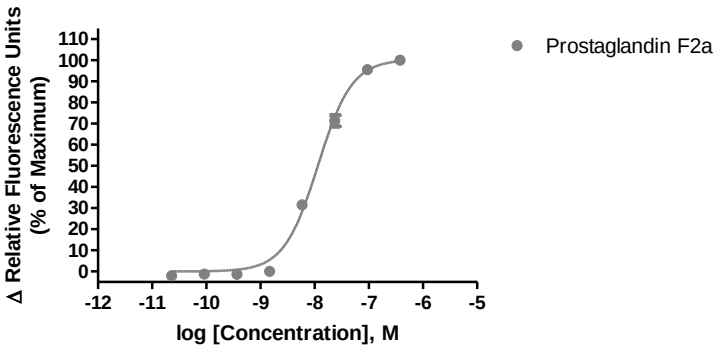

Figure 1. Representative data for activation of FP receptor. Calcium flux in FP–expressing Chem-1 cell line induced by Prostaglandin F2a. FP–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,000 RLU (Relative Light Units).

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin F2a	Calcium Flux	7.4	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
Prostaglandin F2a ligand	Cayman: 16010
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.

EXONGENOUS GENE EXPRESSION

PTGFR cDNA (Accession Number: NM_000959; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG TCC ATG AAC AAT TCC AAA CAG CTA GTG TCT CCT
                                                 M   S   M   N   N   S   K   Q   L   V   S   P

GCA GCT GCG CTT CTT TCA AAC ACA ACC TGC CAG ACG GAA AAC CGG CTT TCC GTA TTT TTT TCA GTA ATC
 A   A   A   L   L   S   N   T   T   C   Q   T   E   N   R   L   S   V   F   F   S   V   I

TTC ATG ACA GTG GGA ATC TTG TCA AAC AGC CTT GCC ATC GCC ATT CTC ATG AAG GCA TAT CAG AGA TTT
 F   M   T   V   G   I   L   S   N   S   L   A   I   A   I   L   M   K   A   Y   Q   R   F

AGA CAG AAG TCC AAG GCA TCG TTT CTG CTT TTG GCC AGT GGC CTG GTA ATC ACT GAT TTC TTT GGC CAT
 R   Q   K   S   K   A   S   F   L   L   L   A   S   G   L   V   I   T   D   F   F   G   H

CTC ATC AAT GGA GCC ATA GCA GTA TTT GTA TAT GCT TCT GAT AAA GAA TGG ATC CGC TTT GAC CAA TCA
 L   I   N   G   A   I   A   V   F   V   Y   A   S   D   K   E   W   I   R   F   D   Q   S

AAT GTC CTT TGC AGT ATT TTT GGT ATC TGC ATG GTG TTT TCT GGT CTG TGC CCA CTT CTT CTA GGC AGT
 N   V   L   C   S   I   F   G   I   C   M   V   F   S   G   L   C   P   L   L   L   G   S

GTG ATG GCC ATT GAG CGG TGT ATT GGA GTC ACA AAA CCA ATA TTT CAT TCT ACG AAA ATT ACA TCC AAA
 V   M   A   I   E   R   C   I   G   V   T   K   P   I   F   H   S   T   K   I   T   S   K

CAT GTG AAA ATG ATG TTA AGT GGT GTG TGC TTG TTT GCT GTT TTC ATA GCT TTG CTG CCC ATC CTT GGA
 H   V   K   M   M   L   S   G   V   C   L   F   A   V   F   I   A   L   L   P   I   L   G

CAT CGA GAC TAT AAA ATT CAG GCG TCG AGG ACC TGG TGT TTC TAC AAC ACA GAA GAC ATC AAA GAC TGG
 H   R   D   Y   K   I   Q   A   S   R   T   W   C   F   Y   N   T   E   D   I   K   D   W

GAA GAT AGA TTT TAT CTT CTA CTT TTT TCT TTT CTG GGG CTC TTA GCC CTT GGT GTT TCA TTG TTG TGC
 E   D   R   F   Y   L   L   L   F   S   F   L   G   L   L   A   L   G   V   S   L   L   C

AAT GCA ATC ACA GGA ATT ACA CTT TTA AGA GTT AAA TTT AAA AGT CAG CAG CAC AGA CAA GGC AGA TCT
 N   A   I   T   G   I   T   L   L   R   V   K   F   K   S   Q   Q   H   R   Q   G   R   S

CAT CAT TTG GAA ATG GTA ATC CAG CTC CTG GCG ATA ATG TGT GTC TCC TGT ATT TGT TGG AGC CCA TTT
 H   H   L   E   M   V   I   Q   L   L   A   I   M   C   V   S   C   I   C   W   S   P   F

CTG GTT ACA ATG GCC AAC ATT GGA ATA AAT GGA AAT CAT TCT CTG GAA ACC TGT GAA ACA ACA CTT TTT
 L   V   T   M   A   N   I   G   I   N   G   N   H   S   L   E   T   C   E   T   T   L   F

GCT CTC CGA ATG GCA ACA TGG AAT CAA ATC TTA GAT CCT TGG GTA TAT ATT CTT CTA CGA AAG GCT GTC
 A   L   R   M   A   T   W   N   Q   I   L   D   P   W   V   Y   I   L   L   R   K   A   V

CTT AAG AAT CTC TAT AAG CTT GCC AGT CAA TGC TGT GGA GTG CAT GTC ATC AGC TTA CAT ATT TGG GAG
 L   K   N   L   Y   K   L   A   S   Q   C   C   G   V   H   V   I   S   L   H   I   W   E

CTT AGT TCC ATT AAA AAT TCC TTA AAG GTT GCT GCT ATT TCT GAG TCA CCA GTT GCA GAG AAA TCA GCA
 L   S   S   I   K   N   S   L   K   V   A   A   I   S   E   S   P   V   A   E   K   S   A

AGC ACC TGA
 S   T   Stp
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Crowston JG *et al.* (2004) Effect of latanoprost on intraocular pressure in mice lacking the prostaglandin FP receptor. *Invest. Ophthalmol. Vis. Sci.* 45: 3555-9.
2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

3. Sugimoto Y *et al.* (1997) Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277: 681-684.
4. Takayama K *et al.* (2005) Thromboxane A2 and prostaglandin F_2. mediate inflammatory tachycardia. *Nat. Med.* 11: 562-566.

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