eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ FP Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS093LRTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial.

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.

ChemiBrite™ cells express a novel variant of clytin, a calcium-activated photoprotein, to enable sensitive luminescent detection of ligand-induced calcium flux. The ChemiBrite™ version of clytin contains a mutation that increases its affinity for calcium to a level that permits detection of cytosolic calcium in many cells with greater sensitivity than other photoproteins targeted to the mitochondria. Luminescent calcium assays offer several advantages over fluorescent calcium assays including increased sensitivity and lack of interference from fluorescent compounds.

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 PGF2□ binds specifically to the FP receptor, which couples to Gq/11 to mobilize intracellular calcium. Binding of PGF2□ to FP receptors in the corpus luteum is required for luteolysis and subsequent parturition in mice (Sugimoto et al., 1998). PGF2□ also decreases intraocular pressure by an FP-dependent mechanism, and an PGF2□□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). Cloned FP receptor-expressing ChemiBrite™ cells were made by stable transfection of HEK293 cells with ChemiBrite™ clytin and the FP receptor. These stability tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the FP receptor.

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: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

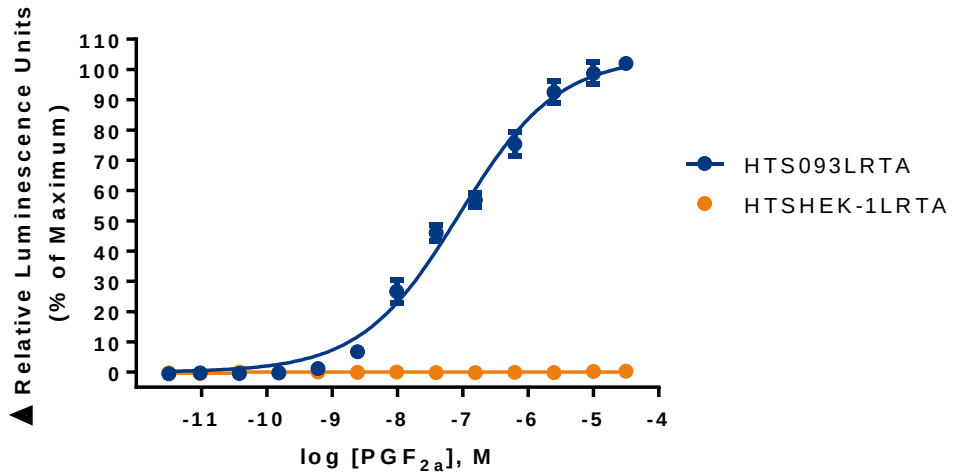

Figure 1. Representative data for activation of FP receptor stably expressed in HEK293 cells induced by PGF2a using a luminescent calcium flux assay. FP–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPRTETRA® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 190,000 RLU (Relative Light Units) as measured by AUC (area under curve) for 80 s post agonist addition using the provided protocol. Similarly parental cells (catalog # HTSHEK-1L were tested to determine the specificity of the resulting signal.

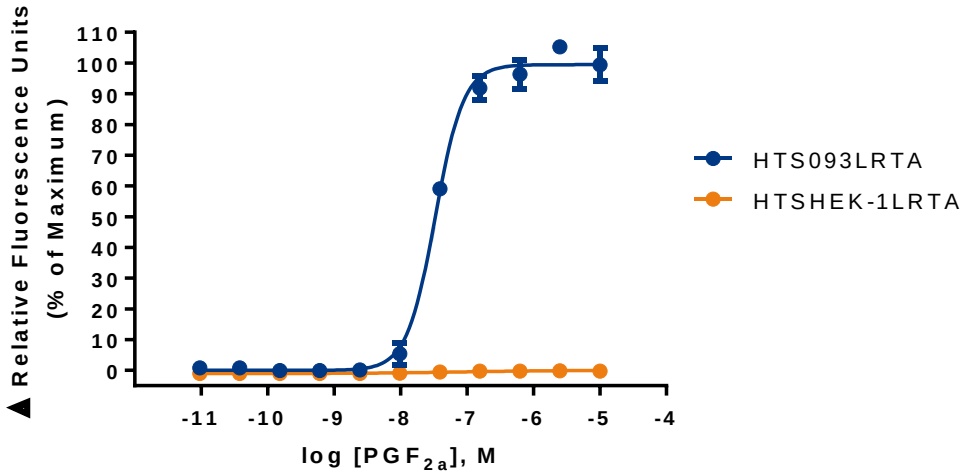

Figure 2. Representative data for activation of FP receptor stably expressed in HEK293 cells induced by PGF2a using a fluorescent calcium flux assay. FP–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPRTETRA® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 13,000 RLU as measured by Maximal Response for 20s post agonist addition. Similarly parental cells (catalog # HTSHEK-1L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of FP-expressing HEK293 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin F2a	Calcium Flux - Luminescence	48.0	Eurofins Internal Data
Prostaglandin F2a	Calcium Flux - Fluorescence	33.0	Eurofins Internal Data

ASSAY SETUP

Luminescence

Table 2. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Luminescence
Ex/Em	None/None
Camera Gain	280,000
Gate Open	100 %
Exposure Time	0.9 sec
Read Interval	1 sec.
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 3. Luminescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Quest Fluo-8™, AM	AAT Bioquest: 21080
PGF_{2a} ligand	Cayman: 16010
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

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. Fluorescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
PGF_{2a} ligand	Cayman: 16010
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol – Luminescence

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 18-24 h.
9. Next day, prepare Assay buffer (HBSS, 20mM HEPES, pH 7.4) and Loading buffer (Assay buffer with 10µM coelenterazine). *Note: Please prepare coelenterazine stock according to Manufacturer's Recommendations at 10mM to allow for 1:1000 dilution into Loading buffer (10uM final concentration). It is critical that coelenterazine solution is prepared at room temperature and is protected from light.*
10. Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
11. 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.
12. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
13. 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.
14. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

Assay Protocol – Fluorescence

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. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
8. 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
9.	Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
10.	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.
11.	Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
12.	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.
13.	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

HEK293

EXONGENOUS GENE EXPRESSION

Human PTGFR cDNA (Accession Number: NM_000959; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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ATG TCC ATG AAC AAT TCC AAA CAG CTA GTG TCT CCT GCA GCT GCG CTT CTT TCA AAC ACA ACC TGC CAG
 M   S   M   N   N   S   K   Q   L   V   S   P   A   A   A   L   L   S   N   T   T   C   Q

ACG GAA AAC CGG CTT TCC GTA TTT TTT TCA GTA ATC TTC ATG ACA GTG GGA ATC TTG TCA AAC AGC CTT
 T   E   N   R   L   S   V   F   F   S   V   I   F   M   T   V   G   I   L   S   N   S   L

GCC ATC GCC ATT CTC ATG AAG GCA TAT CAG AGA TTT AGA CAG AAG TCC AAG GCA TCG TTT CTG CTT TTG
 A   I   A   I   L   M   K   A   Y   Q   R   F   R   Q   K   S   K   A   S   F   L   L   L

GCC AGT GGC CTG GTA ATC ACT GAT TTC TTT GGC CAT CTC ATC AAT GGA GCC ATA GCA GTA TTT GTA TAT
 A   S   G   L   V   I   T   D   F   F   G   H   L   I   N   G   A   I   A   V   F   V   Y

GCT TCT GAT AAA GAA TGG ATC CGC TTT GAC CAA TCA AAT GTC CTT TGC AGT ATT TTT GGT ATC TGC ATG
 A   S   D   K   E   W   I   R   F   D   Q   S   N   V   L   C   S   I   F   G   I   C   M

GTG TTT TCT GGT CTG TGC CCA CTT CTT CTA GGC AGT GTG ATG GCC ATT GAG CGG TGT ATT GGA GTC ACA
 V   F   S   G   L   C   P   L   L   L   G   S   V   M   A   I   E   R   C   I   G   V   T

AAA CCA ATA TTT CAT TCT ACG AAA ATT ACA TCC AAA CAT GTG AAA ATG ATG TTA AGT GGT GTG TGC TTG
 K   P   I   F   H   S   T   K   I   T   S   K   H   V   K   M   M   L   S   G   V   C   L

TTT GCT GTT TTC ATA GCT TTG CTG CCC ATC CTT GGA CAT CGA GAC TAT AAA ATT CAG GCG TCG AGG ACC
 F   A   V   F   I   A   L   L   P   I   L   G   H   R   D   Y   K   I   Q   A   S   R   T

TGG TGT TTC TAC AAC ACA GAA GAC ATC AAA GAC TGG GAA GAT AGA TTT TAT CTT CTA CTT TTT TCT TTT
 W   C   F   Y   N   T   E   D   I   K   D   W   E   D   R   F   Y   L   L   L   F   S   F

CTG GGG CTC TTA GCC CTT GGT GTT TCA TTG TTG TGC AAT GCA ATC ACA GGA ATT ACA CTT TTA AGA GTT
 L   G   L   L   A   L   G   V   S   L   L   C   N   A   I   T   G   I   T   L   L   R   V

AAA TTT AAA AGT CAG CAG CAC AGA CAA GGC AGA TCT CAT CAT TTG GAA ATG GTA ATC CAG CTC CTG GCG
K   F   K   S   Q   Q   H   R   Q   G   R   S   H   H   L   E   M   V   I   Q   L   L   A

ATA ATG TGT GTC TCC TGT ATT TGT TGG AGC CCA TTT CTG GTT ACA ATG GCC AAC ATT GGA ATA AAT GGA
 I   M   C   V   S   C   I   C   W   S   P   F   L   V   T   M   A   N   I   G   I   N   G

AAT CAT TCT CTG GAA ACC TGT GAA ACA ACA CTT TTT GCT CTC CGA ATG GCA ACA TGG AAT CAA ATC TTA
 N   H   S   L   E   T   C   E   T   T   L   F   A   L   R   M   A   T   W   N   Q   I   L

GAT CCT TGG GTA TAT ATT CTT CTA CGA AAG GCT GTC CTT AAG AAT CTC TAT AAG CTT GCC AGT CAA TGC
 D   P   W   V   Y   I   L   L   R   K   A   V   L   K   N   L   Y   K   L   A   S   Q   C

TGT GGA GTG CAT GTC ATC AGC TTA CAT ATT TGG GAG CTT AGT TCC ATT AAA AAT TCC TTA AAG GTT GCT
 C   G   V   H   V   I   S   L   H   I   W   E   L   S   S   I   K   N   S   L   K   V   A

GCT ATT TCT GAG TCA CCA GTT GCA GAG AAA TCA GCA AGC ACC TGA
 A   I   S   E   S   P   V   A   E   K   S   A   S   T  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-1L	ChemiBrite™ HEK stable cell line (control cells)
HTS081L	ChemiBrite™ TP Prostanoid family Stable Cell Line
HTS081LRTA	Ready-to-Assay™ ChemiBrite™ TP Prostanoid Receptor Frozen Cells
HTS185L	ChemiBrite™ HEK stable EP_2 Prostanoid Receptor Cell Line
HTS185LRTA	Ready-to-Assay™ ChemiBrite™ EP_2 Prostanoid Receptor Frozen Cells
HTS142L	ChemiBrite™ HEK stable EP_4 Prostanoid Receptor Cell Line
HTS142LRTA	Ready-to-Assay™ ChemiBrite™ EP_4 Prostanoid Receptor Frozen Cells
HTS091L	ChemiBrite™ HEK stable DP Prostanoid Receptor Cell Line
HTS091LRTA	Ready-to-Assay™ ChemiBrite™ DP Prostanoid Receptor Frozen Cells
HTS092L	ChemiBrite™ HEK stable EP_3 Prostanoid Receptor Cell Line
HTS092LRTA	Ready-to-Assay™ ChemiBrite™ EP_3 Prostanoid Receptor Frozen Cells
HTS099L	ChemiBrite™ HEK stable EP_1 Prostanoid Receptor Cell Line
HTS099LRTA	Ready-to-Assay™ ChemiBrite™ EP_1 Prostanoid Receptor Frozen Cells
HTS131L	ChemiBrite™ HEK stable IP1 Prostanoid Receptor Cell Line
HTS131LRTA	Ready-to-Assay™ ChemiBrite™ IP1 Prostanoid Receptor Frozen Cells
HTS093L	ChemiBrite™ HEK stable FP Prostanoid Receptor Cell Line
HTS093RTA	Ready-to-Assay™ FP Prostanoid receptor frozen cells
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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