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PRODUCT DATASHEET

ChemiBrite™ EP_3 Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS092L

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

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 PGE_2 causes pain, vasodilatation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP_1, EP_2, EP_3 and EP_4, each bind to PGE_2, but the different G protein coupling status of each receptor leads to distinct biological effects. Further diversity is generated by alternative splicing; the human gene for EP_3 generates 9 alternatively- spliced mRNAs encoding 8 isoforms of EP_3 (Kotani *et al.*, 1997). These isoforms of EP_3 vary in sequence at their C-termini, and differ in their ability to couple to G_s, G_q or G_i (Kotani *et al.*, 1995). EP_3 receptor activity is required for fever induced by pyrogens, a response long attributed to prostaglandins by the antipyretic action of aspirin and other COX inhibitors (Ushikubi *et al.*, 1998). In animal models of allergy, PGE_2-mediated activation of EP_3 inhibits inflammation to counteract the allergy-promoting activity of PGD_2 (Kunikata *et al.*, 2005) Cloned EP_3 receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin, the EP_3 receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the EP_3 receptor.

USE RESTRICTIONS

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WARNINGS

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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

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APPLICATIONS

Calcium Flux Assays; Luminescent Mode and Fluorescent Mode

APPLICATION DATA

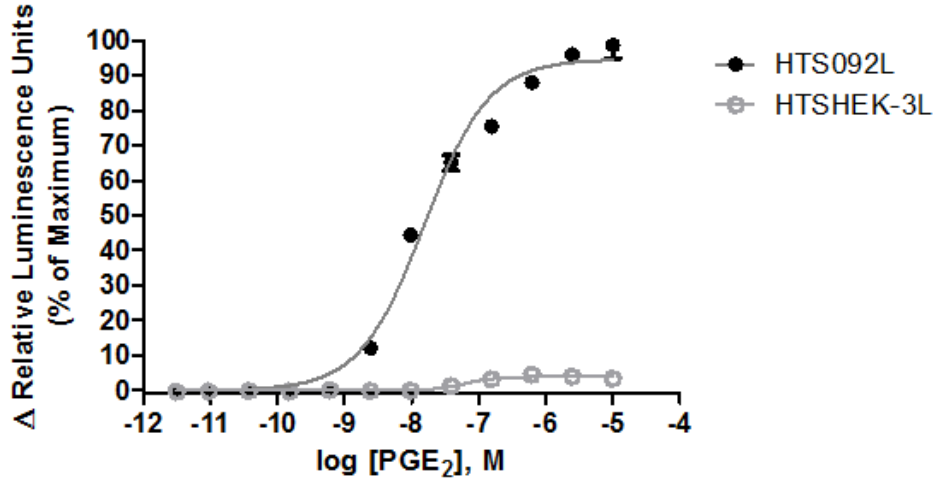

Figure 1. Representative data for activation of EP_3 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a luminescent calcium flux assay. EP_3–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 200,000 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-3L) were tested to determine the specificity of the resulting signal.

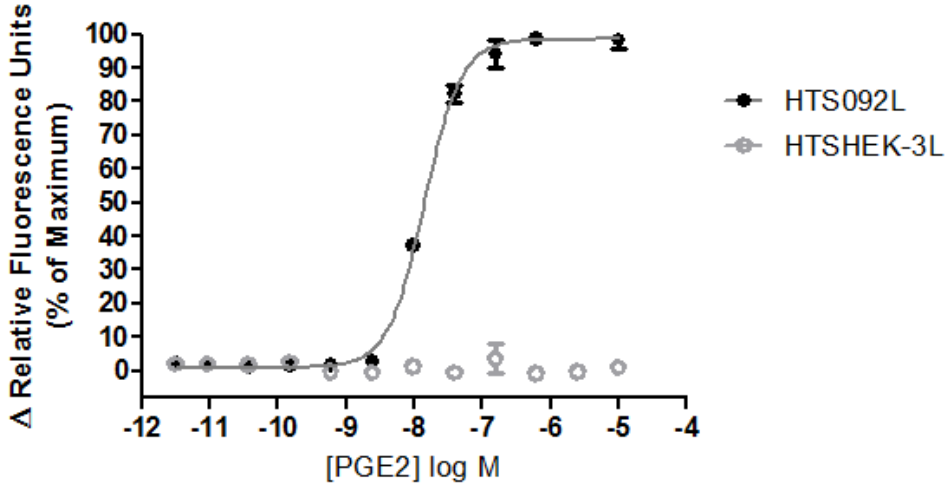

Figure 2. Representative data for activation of EP_3 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a fluorescent calcium flux assay. EP_3–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 6,200 RLU as measured at 10 s post agonist addition. Similarly parental cells (catalog #: HTSHEK-3L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of EP_2-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Prostaglandin E2	Calcium Flux - Luminescence	15*	Eurofins Internal Data
Prostaglandin E2	Calcium Flux - Fluorescence	14	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 luminescence. The Z' value, as defined with response to 10uM Prostaglandin E2 is 0.8.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM F12	-	Millipore: DF041-B
	Fetal Bovine Serum (FBS)	10%	Gibco: 16000
	Non-Essential Amino Acids (NEAA)	1X	Millipore: TMS-001-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	400 µg/ml	Merck EMD: 345812
	Puromycin	1 ug/ml	Merck EMD: 400053
	Hygromycin	200 µg/ml	Merck EMD: 540411
Dissociation	Sterile PBS	-	Millipore: BSS-1006A
	0.05% Trypsin-EDTA	-	Millipore: SM-2002-C
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Gibco: 16000
	Dimethyl Sulfoxide (DMSO)	10%	Merck EMD: 317275

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 2 min, RT, 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	2.0	24
T75	15	1.0	48
T75	15	0.8	72
T150	30	4.0	24
T150	30	3.0	48
T150	30	2.0	72

ASSAY SETUP

Luminescence

Table 4. 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 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
PGE2 ligand	Cayman: 14010
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 – Luminescence

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, 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.*
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 4. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

HOST CELL

HEK293

EXOGENOUS GENE EXPRESSION

Human PTGER3 cDNA (Accession Number: NM_198716; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein and promiscuous G protein expressed in a bicistronic vector

CODING SEQUENCE

```
ATG AAG GAG ACC CGG GGC TAC GGA GGG GAT GCC CCC TTC TGC ACC CGC CTC AAC CAC TCC TAC ACA GGC
 M   K   E   T   R   G   Y   G   G   D   A   P   F   C   T   R   L   N   H   S   Y   T   G

ATG TGG GCG CCC GAG CGT TCC GCC GAG GCG CGG GGC AAC CTC ACG CGC CCT CCA GGG TCT GGC GAG GAT
 M   W   A   P   E   R   S   A   E   A   R   G   N   L   T   R   P   P   G   S   G   E   D

TGC GGA TCG GTG TCC GTG GCC TTC CCG ATC ACC ATG CTG CTC ACT GGT TTC GTG GGC AAC GCA CTG GCC
 C   G   S   V   S   V   A   F   P   I   T   M   L   L   T   G   F   V   G   N   A   L   A

ATG CTG CTC GTG TCG CGC AGC TAC CGG CGC CGG GAG AGC AAG CGC AAG AAG TCC TTC CTG CTG TGC ATC
 M   L   L   V   S   R   S   Y   R   R   R   E   S   K   R   K   K   S   F   L   L   C   I

GGC TGG CTG GCG CTC ACC GAC CTG GTC GGG CAG CTT CTC ACC ACC CCG GTC GTC ATC GTC GTG TAC CTG
 G   W   L   A   L   T   D   L   V   G   Q   L   L   T   T   P   V   V   I   V   V   Y   L

TCC AAG CAG CGT TGG GAG CAC ATC GAC CCG TCG GGG CGG CTC TGC ACC TTT TTC GGG CTG ACC ATG ACT
 S   K   Q   R   W   E   H   I   D   P   S   G   R   L   C   T   F   F   G   L   T   M   T

GTT TTC GGG CTC TCC TCG TTG TTC ATC GCC AGC GCC ATG GCC GTC GAG CGG GCG CTG GCC ATC AGG GCG
 V   F   G   L   S   S   L   F   I   A   S   A   M   A   V   E   R   A   L   A   I   R   A

CCG CAC TGG TAT GCG AGC CAC ATG AAG ACG CGT GCC ACC CGC GCT GTG CTG CTC GGC GTG TGG CTG GCC
 P   H   W   Y   A   S   H   M   K   T   R   A   T   R   A   V   L   L   G   V   W   L   A

GTG CTC GCC TTC GCC CTG CTG CCG GTG CTG GGC GTG GGC CAG TAC ACC GTC CAG TGG CCC GGG ACG TGG
 V   L   A   F   A   L   L   P   V   L   G   V   G   Q   Y   T   V   Q   W   P   G   T   W
```

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TGC TTC ATC AGC ACC GGG CGA GGG GGC AAC GGG ACT AGC TCT TCG CAT AAC TGG GGC AAC CTT TTC TTC
 C   F   I   S   T   G   R   G   G   N   G   T   S   S   S   H   N   W   G   N   L   F   F

GCC TCT GCC TTT GCC TTC CTG GGG CTC TTG GCG CTG ACA GTC ACC TTT TCC TGC AAC CTG GCC ACC ATT
 A   S   A   F   A   F   L   G   L   L   A   L   T   V   T   F   S   C   N   L   A   T   I

AAG GCC CTG GTG TCC CGC TGC CGG GCC AAG GCC ACG GCA TCT CAG TCC AGT GCC CAG TGG GGC CGC ATC
 K   A   L   V   S   R   C   R   A   K   A   T   A   S   Q   S   S   A   Q   W   G   R   I

ACG ACC GAG ACG GCC ATT CAG CTT ATG GGG ATC ATG TGC GTG CTG TCG GTC TGC TGG TCT CCG CTC CTG
 T   T   E   T   A   I   Q   L   M   G   I   M   C   V   L   S   V   C   W   S   P   L   L

ATA ATG ATG TTG AAA ATG ATC TTC AAT CAG ACA TCA GTT GAG CAC TGC AAG ACA CAC ACG GAG AAG CAG
 I   M   M   L   K   M   I   F   N   Q   T   S   V   E   H   C   K   T   H   T   E   K   Q

AAA GAA TGC AAC TTC TTC TTA ATA GCT GTT CGC CTG GCT TCA CTG AAC CAG ATC TTG GAT CCT TGG GTT
 K   E   C   N   F   F   L   I   A   V   R   L   A   S   L   N   Q   I   L   D   P   W   V

TAC CTG CTG TTA AGA AAG ATC CTT CTT CGA AAG TTT TGC CAG ATG AGA AAA AGA AGA CTC AGA GAG CAA
 Y   L   L   L   R   K   I   L   L   R   K   F   C   Q   M   R   K   R   R   L   R   E   Q

GAG GAA TTT TGG GGA AAT TGA
 E   E   F   W   G   N  Stp
```

RELATED PRODUCTS

Product Number	Description
HTSHEK-1L	ChemiBrite™ HEK stable cell line (control cells)
HTSHEK-3L	ChemiBrite™ HEK Gαqi stable cell line (control cells)
HTS185L	ChemiBrite™ HEK stable EP2 Prostanoid Receptor Cell Line
HTS142L	ChemiBrite™ HEK stable EP4 Prostanoid Receptor Cell Line
HTS081L	ChemiBrite™ HEK stable TP Prostanoid Receptor Cell Line
HTS091L	ChemiBrite™ HEK stable DP Prostanoid Receptor Cell Line
HTS093L	ChemiBrite™ HEK stable FP Prostanoid Receptor Cell Line
HTS092RTA	Ready-to-Assay ™ EP3 Prostanoid receptor frozen cells
HTS092M	ChemiScreen™ EP3 Prostanoid receptor Membrane Prep
HTSHEK-1L	ChemiBrite™ HEK stable cell line (control cells)

REFERENCES

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2. Kotani M *et al.* (1997) Structural Organization of the Human Prostaglandin EP_3 Receptor Subtype Gene (PTGER3). *Genomics* 40: 425-434

3. Kunikata T *et al*. (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP_3. *Nat. Immunol.* 6: 524-531.

4. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

5. Ushikubi F *et al.* (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. *Nature* 395: 281-284.

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