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PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite EP_3 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS092LRTA

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 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

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.

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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.

APPLICATIONS

Calcium Flux Assays: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

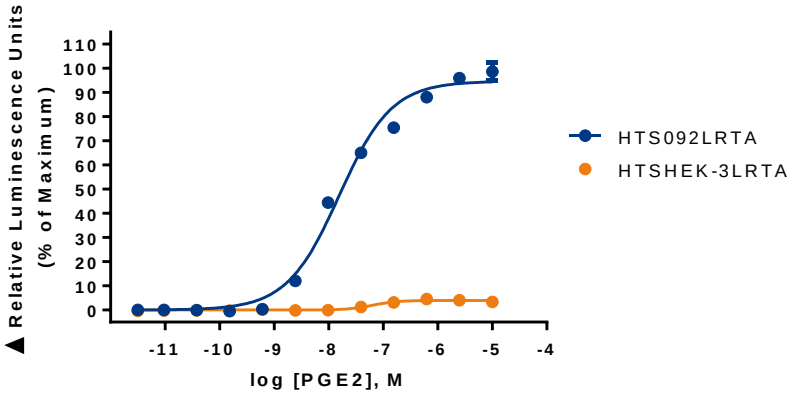

Figure 1. Representative data for activation of EP_3 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a luminescent calcium flux assay. EP_3–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated 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.

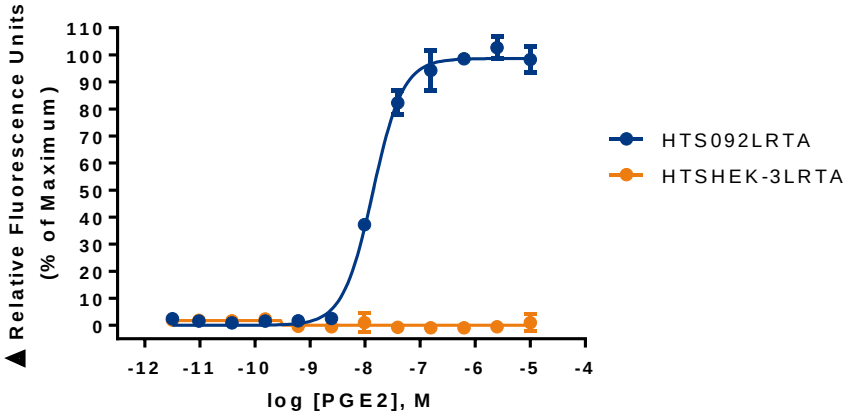

Figure 2. Representative data for activation of EP_3 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a fluorescent calcium flux assay. EP_3–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate the following day the cells were loaded with a calcium 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_3-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

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^{TM}, 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

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
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

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 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

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
HTS185LRTA	Ready-to-Assay™ ChemiBrite™ EP2 Prostanoid Receptor Frozen Cells
HTS142L	ChemiBrite™ HEK stable EP4 Prostanoid Receptor Cell Line
HTS142LRTA	Ready-to-Assay™ ChemiBrite™ EP4 Prostanoid Receptor Frozen Cells
HTS081L	ChemiBrite™ HEK stable TP Prostanoid Receptor Cell Line
HTS081LRTA	Ready-to-Assay™ ChemiBrite™ TP 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 EP3 Prostanoid Receptor Cell Line
HTS093L	ChemiBrite™ HEK stable FP Prostanoid Receptor Cell Line
HTS093LRTA	Ready-to-Assay™ ChemiBrite™ FP Prostanoid Receptor Frozen Cells
HTS092RTA	Ready-to-Assay™ EP3 Prostanoid receptor frozen cells
HTS092M	ChemiScreen™ EP3 Prostanoid receptor Membrane Prep
HTS099L	ChemiBrite™ HEK stable EP1 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

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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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