eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ EP_1 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS099LRTA

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, vasodilation, 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; EP_1 couples primarily to G_q to mobilize intracellular calcium. EP_1 appears to mediate the effects of PGE_2 in promoting formation of precancerous lesions in animal models of colon cancer (Watanabe *et al*., 1999). In addition, EP_1 has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice (Matsuoka *et al*., 2005). Cloned human EP_1 receptor-expressing ChemiBrite™ cells were made by stable transfection of HEK293 cells with ChemiBrite™ clytin and the EP_1 receptor. These stability-tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the EP_1 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

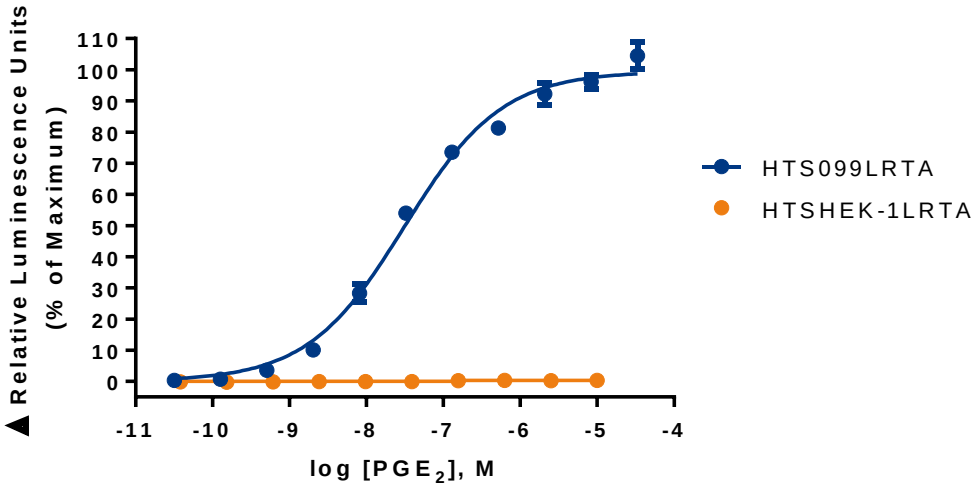

Figure 1. Representative data for activation of EP_1 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a luminescent calcium flux assay. EP_1–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated 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 (are 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.

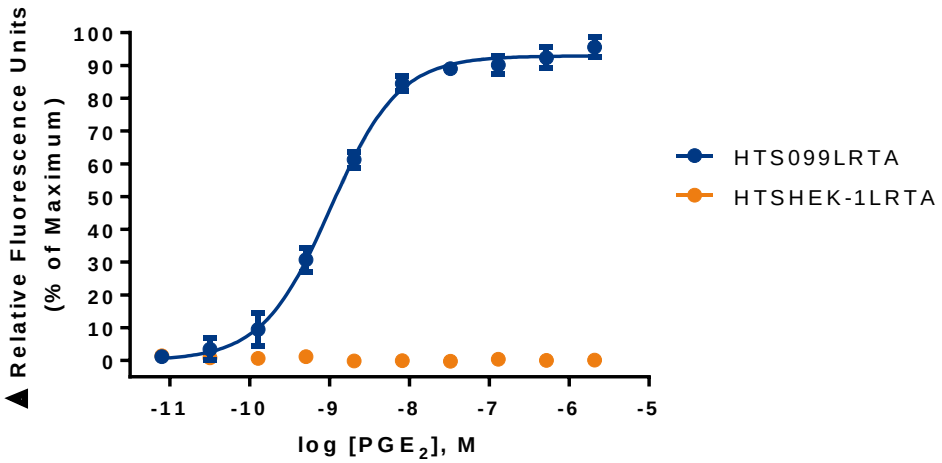

Figure 2. Representative data for activation of EP_1 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a fluorescent calcium flux assay. EP_1–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 12,000 RLU. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of EP_1-expressing HEK293 cells

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
PGE2	Calcium Flux - Fluorescence	16	Eurofins Internal Data
PGE2	Calcium Flux - Luminescence	2.0	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
Prostaglandin E2 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
Prostaglandin E2 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 PTGER1 cDNA (Accession Number: NM_000955; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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ATG AGC CCT TGC GGG CCC CTC AAC CTG AGC CTG GCG GGC GAG GCG ACC ACA TGC GCG GCG CCC TGG GTC CCC AAC
 M   S   P   C   G   P   L   N   L   S   L   A   G   E   A   T   T   C   A   A   P   W   V   P   N

ACG TCG GCC GTG CCG CCG TCG GGC GCT TCG CCC GCG CTG CCC ATC TTC TCC ATG ACG CTG GGC GCC GTG TCC AAC
 T   S   A   V   P   P   S   G   A   S   P   A   L   P   I   F   S   M   T   L   G   A   V   S   N

CTG CTG GCG CTG GCG CTG CTG GCG CAG GCC GCG GGC CGC CTG CGA CGC CGC CGC TCG GCC GCC ACC TTC CTG CTG
 L   L   A   L   A   L   L   A   Q   A   A   G   R   L   R   R   R   R   S   A   A   T   F   L   L

TTC GTG GCC AGC CTG CTG GCC ACC GAC CTG GCG GGC CAC GTG ATC CCG GGC GCG CTG GTG CTG CGT CTG TAC ACT
 F   V   A   S   L   L   A   T   D   L   A   G   H   V   I   P   G   A   L   V   L   R   L   Y   T

GCG GGG CGC GCT CCG GCC GGC GGG GCC TGC CAC TTC CTG GGC GGC TGC ATG GTC TTC TTC GGC CTG TGC CCG CTG
 A   G   R   A   P   A   G   G   A   C   H   F   L   G   G   C   M   V   F   F   G   L   C   P   L

CTG CTG GGC TGT GGC ATG GCC GTG GAG CGC TGC GTG GGC GTC ACG CGG CCG CTG CTC CAC GCC GCG CGG GTC TCG
 L   L   G   C   G   M   A   V   E   R   C   V   G   V   T   R   P   L   L   H   A   A   R   V   S

GTC GCC CGC GCG CGC CTG GCG CTG GCC GCG GTG GCC GCG GTG GCC TTG GCC GTG GCG CTG CTG CCG CTG GCG CGC
 V   A   R   A   R   L   A   L   A   A   V   A   A   V   A   L   A   V   A   L   L   P   L   A   R

GTG GGC CGC TAT GAG CTG CAG TAC CCG GGC ACG TGG TGC TTC ATC GGC CTG GGT CCC CCG GGC GGC TGG CGC CAG
 V   G   R   Y   E   L   Q   Y   P   G   T   W   C   F   I   G   L   G   P   P   G   G   W   R   Q

GCA CTG CTT GCT GGC CTC TTC GCC AGC CTC GGC CTG GTC GCG CTC CTC GCC GCG CTG GTG TGC AAC ACG CTC AGC
 A   L   L   A   G   L   F   A   S   L   G   L   V   A   L   L   A   A   L   V   C   N   T   L   S

GGC CTG GCC CTG CTA CGC GCC CGC TGG CGA CGC CGC TCC CGA CGG CCT CCC CCG GCC TCA GGC CCC GAC AGC CGG
 G   L   A   L   L   R   A   R   W   R   R   R   S   R   R   P   P   P   A   S   G   P   D   S   R

CGT CGC TGG GGG GCG CAC GGA CCC CGC TCG GCC TCC GCC TCG TCC GCC TCG TCC ATC GCT TCG GCC TCC ACC TTC
 R   R   W   G   A   H   G   P   R   S   A   S   A   S   S   A   S   S   I   A   S   A   S   T   F

TTT GGC GGC TCT CGG AGC AGC GGC TCG GCA CGC AGA GCT CGC GCC CAC GAC GTG GAG ATG GTG GGC CAG CTT GTC
 F   G   G   S   R   S   S   G   S   A   R   R   A   R   A   H   D   V   E   M   V   G   Q   L   V

GGT ATC ATG GTG GTG TCG TGC ATC TGC TGG AGC CCA ATG CTG GTG TTG GTG GCG CTG GCC GTC GGC GGC TGG AGC
 G   I   M   V   V   S   C   I   C   W   S   P   M   L   V   L   V   A   L   A   V   G   G   W   S

TCT ACC TCC CTG CAG CGG CCA CTA TTC CTG GCC GTG CGC CTT GCC TCC TGG AAC CAG ATC CTG GAC CCT TGG GTG
 S   T   S   L   Q   R   P   L   F   L   A   V   R   L   A   S   W   N   Q   I   L   D   P   W   V

TAC ATC CTA CTG CGC CAG GCC GTG CTG CGC CAA CTG CTT CGC CTC TTG CCC CCG AGG GCC GGA GCC AAG GGC GGC
 Y   I   L   L   R   Q   A   V   L   R   Q   L   L   R   L   L   P   P   R   A   G   A   K   G   G

CCC GCG GGG CTG GGC CTA ACA CCG AGC GCC TGG GAG GCC AGC TCG CTG CGC AGC TCC CGG CAC AGC GGC CTC AGC
 P   A   G   L   G   L   T   P   S   A   W   E   A   S   S   L   R   S   S   R   H   S   G   L   S
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CAC TTC TGA
 H   F  STP
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RELATED PRODUCTS

Product Number	Description
HTSHEK-1L	ChemiBrite™ HEK293 Parental Stable Cell Line
HTS099RTA	Ready-to-Assay ™ EP1 Prostanoid receptor frozen cells
HTS099M	ChemiScreen™ EP1 Prostanoid receptor Membrane Prep
HTS099L	ChemiBrite™ HEK stable EP1 Prostanoid Receptor Cell Line
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
HTS131L	ChemiBrite™ HEK stable IP1 Prostanoid Receptor Cell Line
HTS131LRTA	Ready-to-Assay™ ChemiBrite™ IP1 Prostanoid Receptor Frozen Cells

REFERENCES

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2. Watanabe K *et al.*(1999) Role of prostaglandin E receptor subtype EP_1 in colon carcinogenesis. *Cancer Res.* 59: 5093-5096.

3. Matsuoka Y et al. (2005) Prostaglandin E receptor EP_1 controls impulsive behavior under stress. *Proc. Natl. Acad. Sci. USA.* 102: 16066-16071.

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