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PRODUCT DATASHEET

ChemiBrite EP_1 Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS099L

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, 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). Millipore's 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

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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.

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APPLICATIONS

Calcium Flux Assays: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

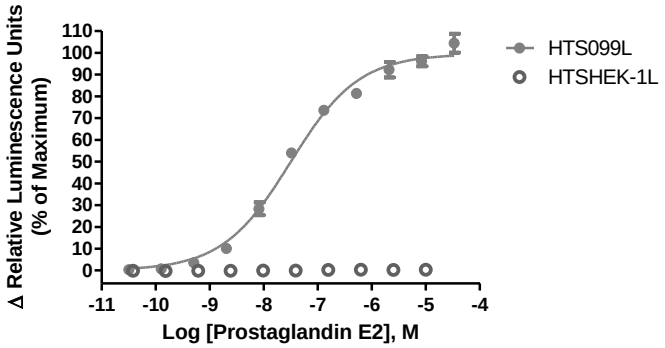

Figure 1. Representative data for activation of EP_1 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a luminescent calcium flux assay. EP_1–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated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

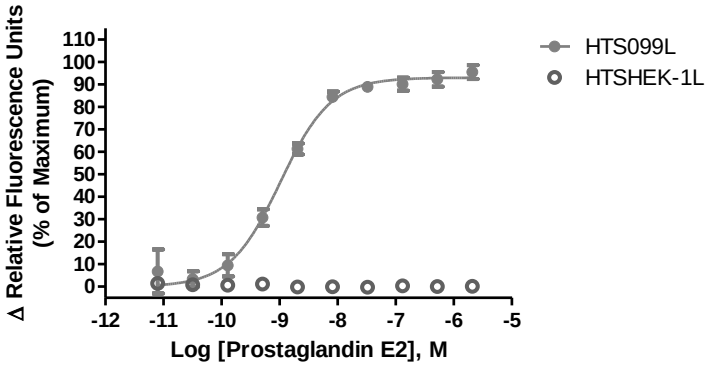

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Figure 2. Representative data for activation of EP_1 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a fluorescent calcium flux assay. EP_1–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, 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

* The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture by calcium flux luminescence. The Z' value, as defined with response to 33uM Prostaglandin is 0.9.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12 Medium	-	Millipore: DF-041-B
	FBS	10%	Gibco: 16000
	Non-Essential Amino Acids (NEAA)	1X	Millipore: TMS-001-C
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 µg/ml	Merck EMD: 400053
	Geneticin (G418)	400 µg/ml	Merck EMD: 345812
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 4 min, 37°C, 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	3.0	24
T75	15	2.0	48
T75	15	1.0	72
T150	30	3.0	24
T150	30	2.0	48
T150	30	1.5	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
BSA (Protease Free). Prepare to 1% in H2O, filter	Merck EMD: 126609
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 (250µg). Prepare to 10mM	Merck EMD: 233900

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, 0.1% BSA, 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 by inverting and tapping/flicking plate. Blot plate.
8.	Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 3 h at room temperature, protected from light.
9.	Prepare ligands in assay buffer at 3x final concentration in non-binding plates.
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 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
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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

CAC TTC TGA
 H   F  STP
```

RELATED PRODUCTS

Product Number	Description
HTSHEK-1L	ChemiBrite™ HEK293 Parental Stable Cell Line
HTS185L	ChemiBrite™ HEK stable EP_2 Prostanoid Receptor Cell Line
HTS009L	ChemiBrite™ HEK stable EP_3 Prostanoid Receptor Cell Line
HTS142L	ChemiBrite™ HEK stable EP_4 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
HTS099RTA	Ready-to-Assay ™ EP_1 Prostanoid receptor frozen cells
HTS099M	ChemiScreen™ EP_1 Prostanoid receptor Membrane Prep

REFERENCES

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

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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