eurofins | Discovery Services

PRODUCT DATASHEET

ChemiBrite™ EP_2 Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS185L

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 bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostanoid PGE2 causes pain, vasodilation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP1, EP2, EP3 and EP4, each bind to PGE2, but the different G protein coupling status of each receptor leads to distinct biological effects. EP2 couples primarily to Gs to increase intracellular cAMP levels. Mice deficient in EP2 receptor showed impaired ovulation and fertilization, salt-sensitive hypertension (Kennedy et al., 1999). It has been shown that EP2 receptors are also involved in cancer associated immunodeficiency. Thus, genetic knockout of the EP2 receptor reduced tumor growth and prolonged survival in mice that had undergone isograft injection of MC26 or Lewis lung carcinoma cells (Yang et al., 2003). The cloned human EP2 receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin, the EP2 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 EP2 receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 ; cAMP Accumulation

APPLICATION DATA

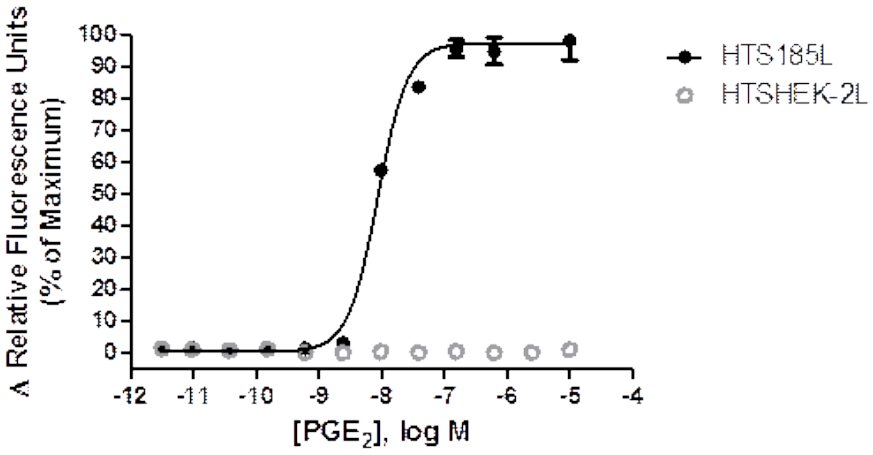

Figure 1. Representative data for activation of EP_2 receptor stably expressed in HEK293 cells induced by Prostaglandin E2 using a fluorescent calcium flux assay. EP_2–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. 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-2L) were tested to determine the specificity of the resulting signal.

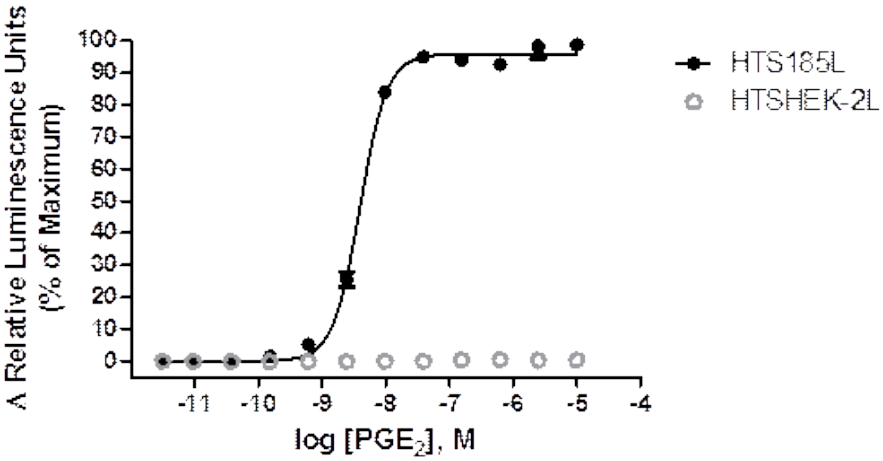

Figure 2. Representative data for activation of EP_2 receptor expressed in HEK293 cells induced by Prostaglandin E2 using a luminescent calcium flux assay. EP_2–expressing HEK293 cells were loaded with 10µM coelenterazine for 3h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 400,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

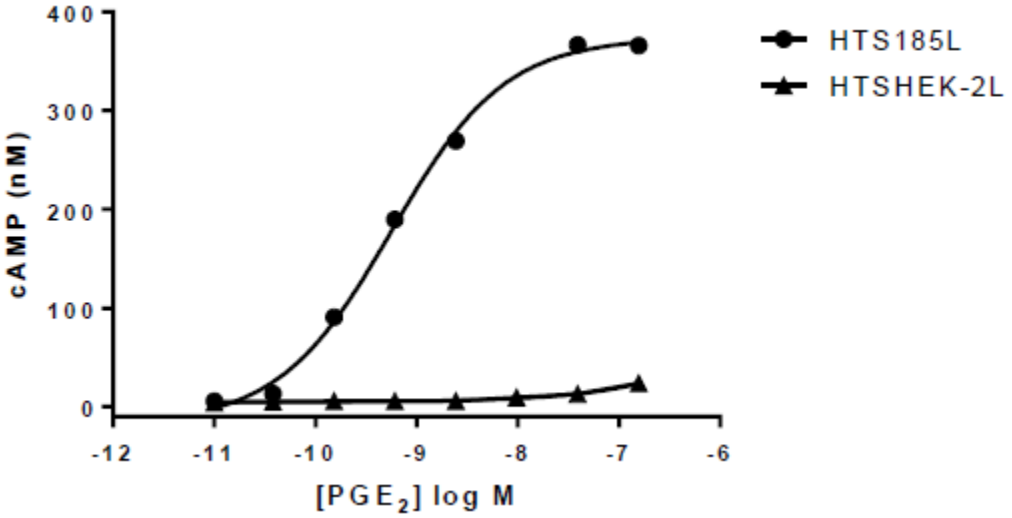

Figure 3. Representative data for activation of EP_2 receptor stably expressed in HEK293 cells induced by PGE2 using a cAMP accumulation assay. EP_2–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with PGE2 for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal fluorescence signal obtained in this experiment was 400 nmoles cAMP/well. Similarly parental cells (catalog #: HTSHEK-2L) 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 - Fluorescence	8.0	Eurofins Internal Data
Prostaglandin E2	Calcium Flux - Luminescence	4.0	Eurofins Internal Data
Prostaglandin E2	cAMP Accumulation	6.0	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 fluorescence. The Z' value, as defined with response to 10µM PGE2, was 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%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
Selection Medium	Basal Medium (see above)	-	

	Geneticin (G418)	400 µg/ml	Invivogen: ant-gn-5
	Puromycin	1 µg/ml	Merck EMD 400053
	Hygromycin	200 µg/ml	Invivogen: ant-hg-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.05% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

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	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	Millipore: TMS-003-C
BSA (Protease Free). Prepare to 1% in H_2O, 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 (250ug). 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, 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 2. 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 PTGER2 cDNA (Accession Number: NM000956; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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ATG GGC AAT GCC TCC AAT GAC TCC CAG TCT GAG GAC TGC GAG ACG CGA CAG TGG CTT CCC CCA GGC GAA
 M   G   N   A   S   N   D   S   Q   S   E   D   C   E   T   R   Q   W   L   P   P   G   E

AGC CCA GCC ATC AGC TCC GTC ATG TTC TCG GCC GGG GTG CTG GGG AAC CTC ATA GCA CTG GCG CTG CTG
 S   P   A   I   S   S   V   M   F   S   A   G   V   L   G   N   L   I   A   L   A   L   L

GCG CGC CGC TGG CGG GGG GAC GTG GGG TGC AGC GCC GGC CGC AGG AGC TCC CTC TCC TTG TTC CAC GTG
 A   R   R   W   R   G   D   V   G   C   S   A   G   R   R   S   S   L   S   L   F   H   V

CTG GTG ACC GAG CTG GTG TTC ACC GAC CTG CTC GGG ACC TGC CTC ATC AGC CCA GTG GTA CTG GCT TCG
 L   V   T   E   L   V   F   T   D   L   L   G   T   C   L   I   S   P   V   V   L   A   S

TAC GCG CGG AAC CAG ACC CTG GTG GCA CTG GCG CCC GAG AGC CGC GCG TGC ACC TAC TTC GCT TTC GCC
 Y   A   R   N   Q   T   L   V   A   L   A   P   E   S   R   A   C   T   Y   F   A   F   A

ATG ACC TTC TTC AGC CTG GCC ACG ATG CTC ATG CTC TTC GCC ATG GCC CTG GAG CGC TAC CTC TCG ATC
 M   T   F   F   S   L   A   T   M   L   M   L   F   A   M   A   L   E   R   Y   L   S   I

GGG CAC CCC TAC TTC TAC CAG CGC CGC GTC TCG CGC TCC GGG GGC CTG GCC GTG CTG CCT GTC ATC TAT
 G   H   P   Y   F   Y   Q   R   R   V   S   R   S   G   G   L   A   V   L   P   V   I   Y

GCA GTC TCC CTG CTC TTC TGC TCG CTG CCG CTG CTG GAC TAT GGG CAG TAC GTC CAG TAC TGC CCC GGG
 A   V   S   L   L   F   C   S   L   P   L   L   D   Y   G   Q   Y   V   Q   Y   C   P   G

ACC TGG TGC TTC ATC CGG CAC GGG CGG ACC GCT TAC CTG CAG CTG TAC GCC ACC CTG CTG CTG CTT CTC
 T   W   C   F   I   R   H   G   R   T   A   Y   L   Q   L   Y   A   T   L   L   L   L   L

ATT GTC TCG GTG CTC GCC TGC AAC TTC AGT GTC ATT CTC AAC CTC ATC CGC ATG CAC CGC CGA AGC CGG
 I   V   S   V   L   A   C   N   F   S   V   I   L   N   L   I   R   M   H   R   R   S   R

AGA AGC CGC TGC GGA CCT TCC CTG GGC AGT GGC CGG GGC GGC CCC GGG GCC CGC AGG AGA GGG GAA AGG
 R   S   R   C   G   P   S   L   G   S   G   R   G   G   P   G   A   R   R   R   G   E   R

GTG TCC ATG GCG GAG GAG ACG GAC CAC CTC ATT CTC CTG GCT ATC ATG ACC ATC ACC TTC GCC GTC TGC
 V   S   M   A   E   E   T   D   H   L   I   L   L   A   I   M   T   I   T   F   A   V   C

TCC TTG CCT TTC ACG ATT TTT GCA TAT ATG AAT GAA ACC TCT TCC CGA AAG GAA AAA TGG GAC CTC CAA
 S   L   P   F   T   I   F   A   Y   M   N   E   T   S   S   R   K   E   K   W   D   L   Q

GCT CTT AGG TTT TTA TCA ATT AAT TCA ATA ATT GAC CCT TGG GTC TTT GCC ATC CTT AGG CCT CCT GTT
 A   L   R   F   L   S   I   N   S   I   I   D   P   W   V   F   A   I   L   R   P   P   V

CTG AGA CTA ATG CGT TCA GTC CTC TGT TGT CGG ATT TCA TTA AGA ACA CAA GAT GCA ACG CAA ACT TCC
 L   R   L   M   R   S   V   L   C   C   R   I   S   L   R   T   Q   D   A  T→T  Q   T   S

TGT CCT ACA CAG TCA GAT GCC AGT AAA CAG GCT GAC CTT TGA
 C   P   T   Q   S   D   A   S   K   Q   A   D   L  Stp
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RELATED PRODUCTS

Product Number	Description
HTSHEK-2L	ChemiScreen™ HEK293 Parental Cell Line (control cells)
HTS185M	ChemiScreen™ EP_2 Prostanoid family receptor membrane prep

REFERENCES

1. Kennedy CR *et al*.(1999) Salt-sensitivity hypertension and reduced fertility in mice lacking the prostaglandin EP_2 receptor. *Nat. Med.* 5:217-220.

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

3. Yang N *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP_2 receptor. *J. Clin. Invest.* 111: 727–735.

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