eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ EP_4 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS142LRTA

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 co-express a GPCR along with 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 mitochondrially expressed photoproteins. Luminescent calcium assays offer several advantages over fluorescent calcium assays including; lower substrate cost, increased sensitivity, and lack of interference from fluorescent compounds.

Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and further modified 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 PGE2 causes pain, vasodilation, immunosuppression of T cells, bone remodeling 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. EP4 couples primarily to Gs to increase intracellular cAMP levels. During neonatal development, EP4 participates in closure of the ductus arteriosus, a process required for switching circulation from the placenta to the lungs (Nguyen et al., 1997). In addition, EP4 mediates PGE2-induced bone formation by promoting osteoblastogenesis, and selective EP4 agonists are being evaluated as potential treatments for osteoporosis (Yoshida et al., 2002). The cloned EP4 receptor-expressing ChemiBrite™ cells are made by stable transfection of HEK293 cells with ChemiBrite™ clytin, EP4 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 EP4 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.

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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 ; cAMP accumulation

APPLICATION DATA

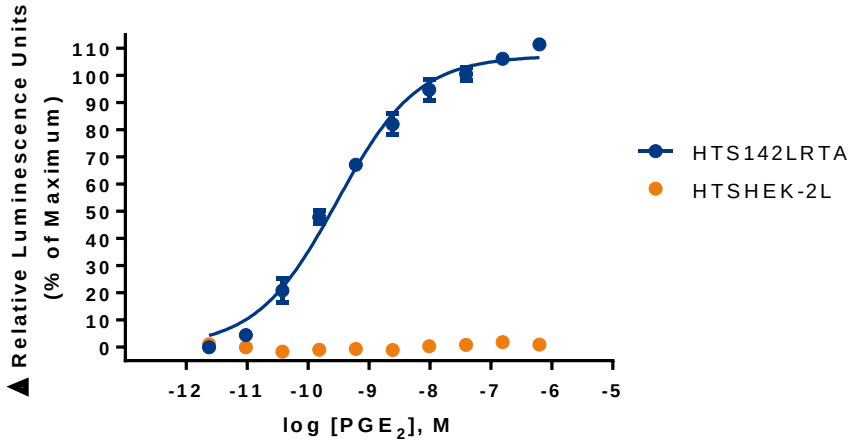

Figure 1. Representative data for activation of EP_4 receptor stably expressed in HEK293 cells induced by PGE_2 using a luminescent calcium flux assay. EP_4–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was 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 240,000 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-2L) were tested to determine the specificity of the resulting signal.

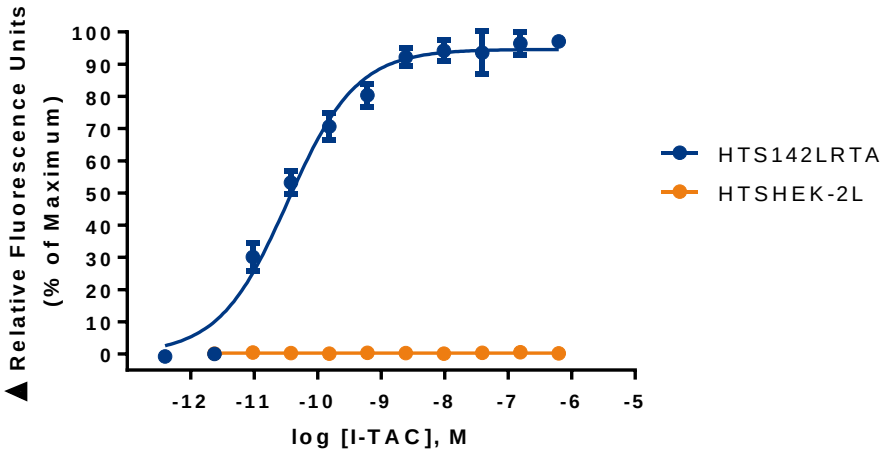

Figure 2. Representative data for activation of EP_4 receptor stably expressed in HEK293 cells induced by PGE_2 using a fluorescent calcium flux assay. EP_4–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, the following day the cells were loaded with a calcium dye, and 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 18,000 RFU. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specific of the resulting signal.

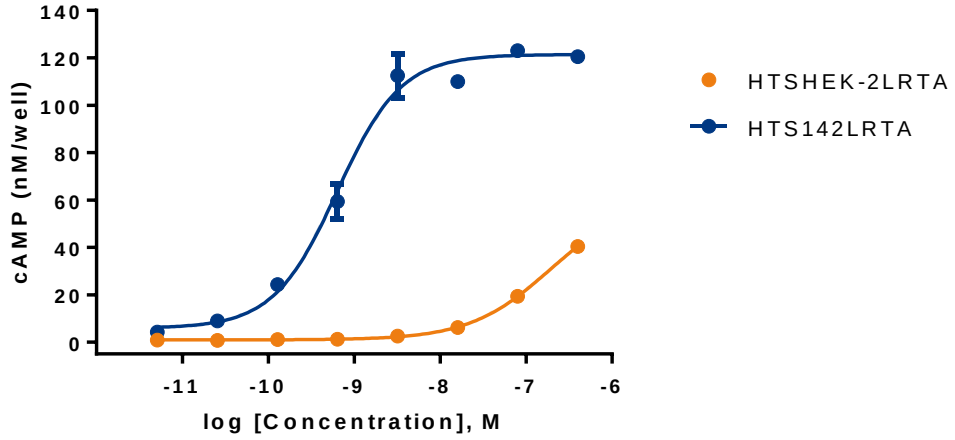

Figure 3. Representative data for activation of EP4 receptor stably expressed in HEK293 cells induced by PGE2 using a cAMP accumulation assay. EP4–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate. The following day the cells were treated with PGE2 for 10 minutes in the presence of 2 mM 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. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of EP_4-expressing HEK293 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
PGE_2	Calcium Flux - Luminescence	0.3	Eurofins Internal Data
PGE_2	Calcium Flux - Fluorescence	0.03	Eurofins Internal Data
PGE_2	cAMP accumulation	0.5	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
PGE_2 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
PGE_2 ligand	Cayman: 14010
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

cAMP

Table 6. Settings for Plate Reader

Option	Setting
Excitation	300 nm
Emission	665/620 nm

Table 7. cAMP Assay Materials (Not provided)

Description	Supplier and Product Number
HEPES 1M Stock	Millipore Sigma: H3537
IBMX Buffer	Sigma #I5879
96-Well Flat Bottom Microtiter Plates	Costar #3917
Non-Binding 96 well Plates (for ligand prep)	Costar: #3789
PGE_2 ligand	Cayman: 14010
cAMP Hi Range Kit	CisBio # 62AM6PEC

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.

Assay Protocol – cAMP

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 20 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.	Prepare Assay Buffer (HBSS, 20mM HEPES, pH 7.4)
10.	Prepare anti-cAMP-Cryptate and cAMP-D2 conjugate stocks. Please prepare stocks according to the Manufacturers Recommendations for reconstitution volume to be used according to Appendix-2 of the insert (Two-step protocol). Mix gently. Store at 10 to 35 °C after reconstitution. Reagent can only go through one cycle of freeze and thaw.
11.	Prepare 1M IBMX Buffer: add 450 µL of DMSO to 1 vial of 100 mg IBMX powder.
12.	Next day, prepare 2mM IBMX Solution: Place the 1M IBMX Buffer and the Assay Buffer in 50-60°C water bath for 10 min to 15 min. Add 10 µL of 1M IBMX Buffer to 4.990 mL of Assay Buffer. Mix by vortex. Place the 2 mM of IBMX Buffer into 34-40°C Incubator for at least 10 minutes or until ready to add to cells.
13.	Prepare 25uL/well of compounds for testing.
14.	Remove the cell assay plate from previous day from cell culture incubator. Quickly invert plate on an absorbent pad and blot to remove all Media Component. Add 25 µL of the 2 mM IBMX Buffer to the plate with the seeded GLP-1/HEK cells. Tap plate gently 3-4 times. Cover plate and incubate inside 34-40°C incubator, static until ready for compound addition.
15.	Add 25 µL compounds, internal control and test sample dilutions to cell assay plate. Cover plate and incubate for 15 to 25 min at 20-25°C
16.	Prepare fresh working dilutions of 1:24 of anti-cAMP-Cryptate and cAMP-D2 conjugate in Lysis Buffer. Protect from light. Do not vortex.
17.	Add 125 µL of cAMP-D2 solution into 2,875 µL of Lysis Buffer for total volume of 3 mL.
18.	Add 125 µL of anti-cAMP-Cryptate solution into 2,875 µL of Lysis Buffer for total volume of 3 mL.
19.	It is imperative that detection reagents are added to plate in the following order: Add 25 µL cAMP-D2 conjugate/lysis buffer solution to each well of cell assay plate. Add 25 µL anti-cAMP-Cryptate/lysis buffer solution to each well of cell assay plate.
20.	Cover with aluminum plate sealer and incubate the cell assay plate 20-25°C, for 60 to 65 min (If available, use gentle plate shaker). Protect from light.
21.	Read plate on a plate reader with 330 nm (excitation) and 665/620 nm (emission).
22.	Calculate Ratio Emission 665/620 nm.

HOST CELL

HEK293

EXONGENOUS GENE EXPRESSION

Human PTGER4 cDNA (Accession Number: NM_000958; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein, and promiscuous G protein, each expressed in a bicistronic vector

CODING SEQUENCE

```
ATG TCC ACT CCC GGG GTC AAT TCG TCC GCC TCC TTG AGC CCC GAC CGG CTG AAC AGC CCA GTG ACC ATC CCG
 M   S   T   P   G   V   N   S   S   A   S   L   S   P   D   R   L   N   S   P   V   T   I   P

GCG GTG ATG TTC ATC TTC GGG GTG GTG GGC AAC CTG GTG GCC ATC GTG GTG CTG TGC AAG TCG CGC AAG GAG
A   V   M   F   I   F   G   V   V   G   N   L   V   A   I   V   V   L   C   K   S   R   K   E

CAG AAG GAG ACG ACC TTC TAC ACG CTG GTA TGT GGG CTG GCT GTC ACC GAC CTG TTG GGC ACT TTG TTG GTG
Q   K   E   T   T   F   Y   T   L   V   C   G   L   A   V   T   D   L   L   G   T   L   L   V

AGC CCG GTG ACC ATC GCC ACG TAC ATG AAG GGC CAA TGG CCC GGG GGC CAG CCG CTG TGC GAG TAC AGC ACC
S   P   V   T   I   A   T   Y   M   K   G   Q   W   P   G   G   Q   P   L   C   E   Y   S   T

TTC ATT CTG CTC TTC TTC AGC CTG TCC GGC CTC AGC ATC ATC TGC GCC ATG AGT GTC GAG CGC TAC CTG GCC
F   I   L   L   F   F   S   L   S   G   L   S   I   I   C   A   M   S   V   E   R   Y   L   A

ATC AAC CAT GCC TAT TTC TAC AGC CAC TAC GTG GAC AAG CGA TTG GCG GGC CTC ACG CTC TTT GCA GTC TAT
 I   N   H   A   Y   F   Y   S   H   Y   V   D   K   R   L   A   G   L   T   L   F   A   V   Y

GCG TCC AAC GTG CTC TTT TGC GCG CTG CCC AAC ATG GGT CTC GGT AGC TCG CGG CTG CAG TAC CCA GAC ACC
 A   S   N   V   L   F   C   A   L   P   N   M   G   L   G   S   S   R   L   Q   Y   P   D   T

TGG TGC TTC ATC GAC TGG ACC ACC AAC GTG ACG GCG CAC GCC GCC TAC TCC TAC ATG TAC GCG GGC TTC AGC
 W   C   F   I   D   W   T   T   N   V   T   A   H   A   A   Y   S   Y   M   Y   A   G   F   S

TCC TTC CTC ATT CTC GCC ACC GTC CTC TGC AAC GTG CTT GTG TGC GGC GCG CTG CTC CGC ATG CAC CGC CAG
 S   F   L   I   L   A   T   V   L   C   N   V   L   V   C   G   A   L   L   R   M   H   R   Q

TTC ATG CGC CGC ACC TCG CTG GGC ACC GAG CAG CAC CAC GCG GCC GCG GCC GCC TCG GTT GCC TCC CGG GGC
 F   M   R   R   T   S   L   G   T   E   Q   H   H   A   A   A   A   A   S   V   A   S   R   G

CAC CCC GCT GCC TCC CCA GCC TTG CCG CGC CTC AGC GAC TTT CGG CGC CGC CGG AGC TTC CGC CGC ATC GCG
 H   P   A   A   S   P   A   L   P   R   L   S   D   F   R   R   R   R   S   F   R   R   I   A

GGC GCC GAG ATC CAG ATG GTC ATC TTA CTC ATT GCC ACC TCC CTG GTG GTG CTC ATC TGC TCC ATC CCG CTC
 G   A   E   I   Q   M   V   I   L   L   I   A   T   S   L   V   V   L   I   C   S   I   P   L

GTG GTG CGA GTA TTC GTC AAC CAG TTA TAT CAG CCA AGT TTG GAG CGA GAA GTC AGT AAA AAT CCA GAT TTG
 V   V   R   V   F   V   N   Q   L   Y   Q   P   S   L   E   R   E   V   S   K   N   P   D     L

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CTC AGT AAA GCA ATA GAG AAG ATC AAA TGC CTC TTC TGC CGC ATT GGC GGG TCC CGC AGG GAG CGC TCC GGA
 L   S   K   A   I   E   K   I   K   C   L   F   C   R   I   G   G   S   R   R   E   R   S   G

CAG CAC TGC TCA GAC AGT CAA AGG ACA TCT TCT GCC ATG TCA GGC CAC TCT CGC TCC TTC ATC TCC CGG GAG
 Q   H   C   S   D   S   Q   R   T   S   S   A   M   S   G   H   S   R   S   F   I   S   R   E

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 L   K   E   I   S   S   T   S   Q   T   L   L   P   D   L   S   L   P   D   L   S   E   N   G

CTT GGA GGC AGG AAT TTG CTT CCA GGT GTG CCT GGC ATG GGC CTG GCC CAG GAA GAC ACC ACC TCA CTG AGG
 L   G   G   R   N   L   L   P   G   V   P   G   M   G   L   A   Q   E   D   T   T   S   L   R

ACT TTG CGA ATA TCA GAG ACC TCA GAC TCT TCA CAG GGT CAG GAC TCA GAG AGT GTC TTA CTG GTG GAT GAG
 T   L   R   I   S   E   T   S   D   S   S   Q   G   Q   D   S   E   S   V   L   L   V   D   E

GCT GGT GGG AGC GGC AGG GCT GGG CCT GCC CCT AAG GGG AGC TCC CTG CAA GTC ACA TTT CCC AGT GAA ACA
 A   G   G   S   G   R   A   G   P   A   P   K   G   S   S   L   Q   V   T   F   P   S   E   T

CTG AAC TTA TCA GAA AAA TGT ATA TGA
 L   N   L   S   E   K   C   I  Stp
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RELATED PRODUCTS

Product Number	Description
HTSHEK-2L	ChemiBrite™ HEK293 stable parental cell line
HTSHEK-1L	ChemiBrite™ HEK stable cell line (control cells)
HTS081L	ChemiBrite™ TP Prostanoid family Stable Cell Line
HTS081LRTA	Ready-to-Assay™ ChemiBrite™ TP Prostanoid Receptor Frozen Cells
HTS185L	ChemiBrite™ HEK stable EP_2 Prostanoid Receptor Cell Line
HTS185LRTA	Ready-to-Assay™ ChemiBrite™ EP_2 Prostanoid Receptor Frozen Cells
HTS142L	ChemiBrite™ HEK stable EP_4 Prostanoid Receptor Cell Line
HTS142LRTA	Ready-to-Assay™ ChemiBrite™ EP_4 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 EP_3 Prostanoid Receptor Cell Line
HTS092LRTA	Ready-to-Assay™ ChemiBrite™ EP_3 Prostanoid Receptor Frozen Cells
HTS093L	ChemiBrite™ HEK stable FP Prostanoid Receptor Cell Line
HTS093LRTA	Ready-to-Assay™ ChemiBrite™ FP Prostanoid Receptor Frozen Cells
HTS099L	ChemiBrite™ HEK stable EP_1 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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4. Cooper et al. (1995) Adenosine Receptor-Induced cyclic AMP generation and inhibition of 5-hydroxytryptamine release in human platelets. *Br J Clin Pharm* 40:43-50

User Agreement (Label License)

In addition to the General Terms and Conditions section, these specific terms also apply for **Ready-to-Assay™ Chemibrite™ EP4 Prostanoid Receptor Frozen Cells, Product No. HTS142LRTA**

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