eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ IP1 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS131LRTA

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.

Prostacyclin (PGI2) is released by vascular endothelial cells and serves as a potent vasodilator, inhibitor of platelet aggregation, and moderator of vascular smooth muscle cell proliferation–migration–differentiation (Narumiya et al. 1999). The function of prostacyclin is mediated via a seven transmembrane GPCR, IP1, which is known to couple to Gs and Gq signaling pathways. Mice lacking the IP1 receptor have shown increased susceptibility to thrombosis (Murata et al. 1997), enhanced injury-induced vascular proliferation and platelet activation (Cheng et al. 2002), as well as reperfusion injury (Xiao et al. 2001). The recent world-wide withdrawal of selective COX-2 inhibitors, rofecoxib (Vioxx™) and valdecoxib (Bextra™) is also due to their discriminating suppression of COX-2-derived prostacyclin and IP1-mediated cardioprotective effects, leading to increased risk of cardiovascular events (Fitzgerald 2004). Cloned human IP1 receptor-expressing ChemiBrite™ cells are made by stable transfection of HEK293 cells with ChemiBrite™ clytin and IP1 Receptor. These stability tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the IP1 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.

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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

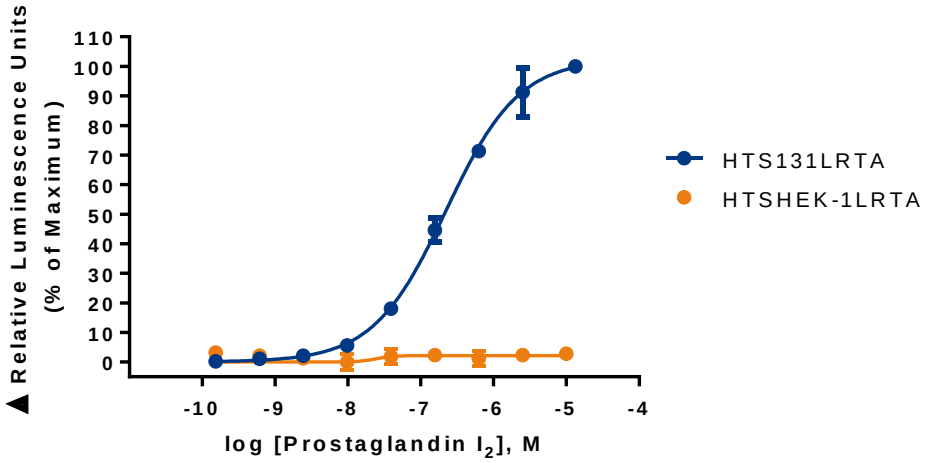

Figure 1. Representative data for activation of IP1 receptor stably expressed in HEK293 cells induced by Prostaglandin I2 using a luminescent calcium flux assay. IP1–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 hrs at room temperature and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPRTETRA® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 30,000 RLU (Relative Light Units) as measured by AUC (are under curve) for 80 sec post agonist addition using the provided protocol. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

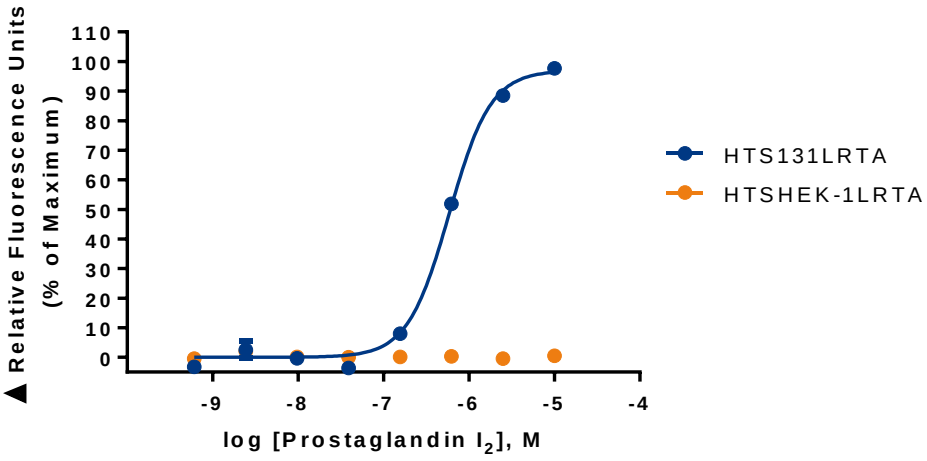

Figure 2. Representative data for activation of IP1 receptor stably expressed in HEK293 cells induced by Prostaglandin I2 using a fluorescent calcium flux assay. IP1–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate. The following day the cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPRTETRA® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 3,000 RLU. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

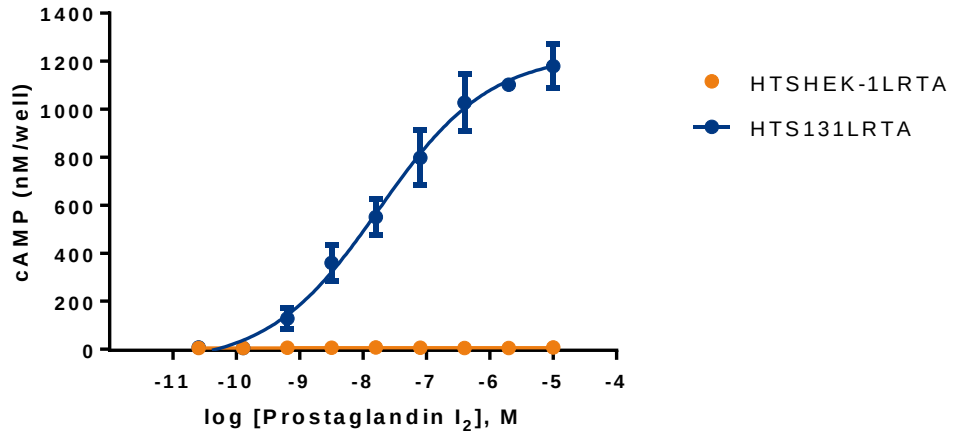

Figure 3. Representative data for activation of IP1 receptor stably expressed in HEK293 cells induced by Prostaglandin I2 using a cAMP accumulation assay. IP1–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with Prostaglandin I2 for 10 minutes in the presence of 100 µM IBMX and 0.5% DMSO to determine receptor mediated cAMP generation using a time-resolve fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of IP_1-expressing HEK293 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin I2	Calcium Flux - Luminescence	222	Eurofins Internal Data
Prostaglandin I2	Calcium Flux - Fluorescence	590	Eurofins Internal Data
Prostaglandin I2	cAMP accumulation	18	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 I2 ligand	Cayman: 18220

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^{TM}, AM	AAT Bioquest: 21080
Prostaglandin I2 ligand	Cayman: 18220
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
Prostaglandin I2 ligand	Cayman: 18220
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

PTGIR cDNA (Accession Number: NM_000960; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein each expressed in a bicistronic vector

CODING SEQUENCE

```
ATG GCG GAT TCG TGC AGG AAC CTC ACC TAC GTG CGG GGC TCG GTG GGG CCG GCC
 M   A   D   S   C   R   N   L   T   Y   V   R   G   S   V   G   P   A

ACC AGC ACC CTG ATG TTC GTG GCC GGT GTG GTG GGC AAC GGG CTG GCC CTG GGC
 T   S   T   L   M   F   V   A   G   V   V   G   N   G   L   A   L   G
```

```
ATC CTG AGC GCA CGG CGA CCG GCG CGC CCC TCG GCC TTC GCG GTG CTG GTC ACC
 I   L   S   A   R   R   P   A   R   P   S   A   F   A   V   L   V   T

GGA CTG GCG GCC ACC GAC CTG CTG GGC ACC AGC TTC CTG AGC CCG GCC GTG TTC
 G   L   A   A   T   D   L   L   G   T   S   F   L   S   P   A   V   F

GTG GCC TAT GCG CGC AAC AGC TCC CTG CTG GGC CTG GCC CGA GGC GGC CCC GCC
 V   A   Y   A   R   N   S   S   L   L   G   L   A   R   G   G   P   A

CTG TGC GAT GCC TTC GCC TTC GCC ATG ACC TTC TTC GGC CTG GCG TCC ATG CTC
 L   C   D   A   F   A   F   A   M   T   F   F   G   L   A   S   M   L

ATC CTC TTT GCC ATG GCC GTG GAG CGC TGC CTG GCG CTG AGC CAC CCC TAC CTC
 I   L   F   A   M   A   V   E   R   C   L   A   L   S   H   P   Y   L

TAC GCG CAG CTG GAC GGG CCC CGC TGC GCC CGC CTG GCG CTG CCA GCC ATC TAC
 Y   A   Q   L   D   G   P   R   C   A   R   L   A   L   P   A   I   Y

GCC TTC TGC GTC CTC TTC TGC GCG CTG CCC CTG CTG GGC CTG GGC CAA CAC CAG
 A   F   C   V   L   F   C   A   L   P   L   L   G   L   G   Q   H   Q

CAG TAC TGC CCC GGC AGC TGG TGC TTC CTC CGC ATG CGC TGG GCC CAG CCG GGC
 Q   Y   C   P   G   S   W   C   F   L   R   M   R   W   A   Q   P   G

GGC GCC GCC TTC TCG CTG GCC TAC GCC GGC CTG GTG GCC CTG CTG GTG GCT GCC
 G   A   A   F   S   L   A   Y   A   G   L   V   A   L   L   V   A   A

ATC TTC CTC TGC AAC GGC TCG GTC ACC CTC AGC CTC TGC CGC ATG TAC CGC CAG
 I   F   L   C   N   G   S   V   T   L   S   L   C   R   M   Y   R   Q

CAG AAG CGC CAC CAG GGC TCT CTG GGT CCA CGG CCG CGC ACC GGA GAG GAC GAG
 Q   K   R   H   Q   G   S   L   G   P   R   P   R   T   G   E   D   E

GTG GAC CAC CTG ATC CTG CTG GCC CTC ATG ACA GTG GTC ATG GCC GTG TGC TCC
 V   D   H   L   I   L   L   A   L   M   T   V   V   M   A   V   C   S

CTG CCT CTC ACG ATC CGC TGC TTC ACC CAG GCT GTC GCC CCT GAC AGC AGC AGT
 L   P   L   T   I   R   C   F   T   Q   A   V   A   P   D   S   S   S

GAG ATG GGG GAC CTC CTT GCC TTC CGC TTC TAC GCC TTC AAC CCC ATC CTG GAC
 E   M   G   D   L   L   A   F   R   F   Y   A   F   N   P   I   L   D

CCC TGG GTC TTC ATC CTT TTC CGC AAG GCT GTC TTC CAG CGA CTC AAG CTC TGG
 P   W   V   F   I   L   F   R   K   A   V   F   Q   R   L   K   L   W

GTC TGC TGC CTG TGC CTC GGG CCT GCC CAC GGA GAC TCG CAG ACA CCC CTT TCC
 V   C   C   L   C   L   G   P   A   H   G   D   S   Q   T   P   L   S

CAG CTC GCC TCC GGG AGG AGG GAC CCA AGG GCC CCC TCT GCT CCT GTG GGA AAG
 Q   L   A   S   G   R   R   D   P   R   A   P   S   A   P   V   G   K

GAG GGG AGC TGC GTG CCT TTG TCG GCT TGG GGC GAG GGG CAG GTG GAG CCC TTG
 E   G   S   C   V   P   L   S   A   W   G   E   G   Q   V   E   P   L

CCT CCC ACA CAG CAG TCC AGC GGC AGC GCC GTG GGA ACG TCG TCC AAA GCA GAA
 P   P   T   Q   Q   S   S   G   S   A   V   G   T   S   S   K   A   E

GCC AGC GTC GCC TGC TCC CTC TGC TGA
 A   S   V   A   C   S   L   C  STP
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
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
HTS131M	ChemiScreen™ IP1 Prostanoid receptor membrane prep
HTS131RTA	Ready-to-Assay™ IP1 Prostanoid receptor frozen cells
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

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5. Fitzgerald GA (2004) Coxibs and cardiovascular disease. *N. Engl. J. Med*. 351: 1709–1711.

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