

PRODUCT DATASHEET

ChemiBrite™ DP Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS091L

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N₂.

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 PGD₂ is produced by mast cells upon activation by allergens, and is present at high levels in allergic diseases. PGD₂ binds to two receptors, DP and CRTH2. DP activates G_s to increase cAMP levels, and lack of DP results in reduced allergic response in animal models of bronchial asthma (Matsuoka et al., 2000). Cloned DP receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin, the DP 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 DP 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.

APPLICATIONS

Calcium Flux Fluorescence Assay, Luminescence Assay, cAMP accumulation

APPLICATION DATA

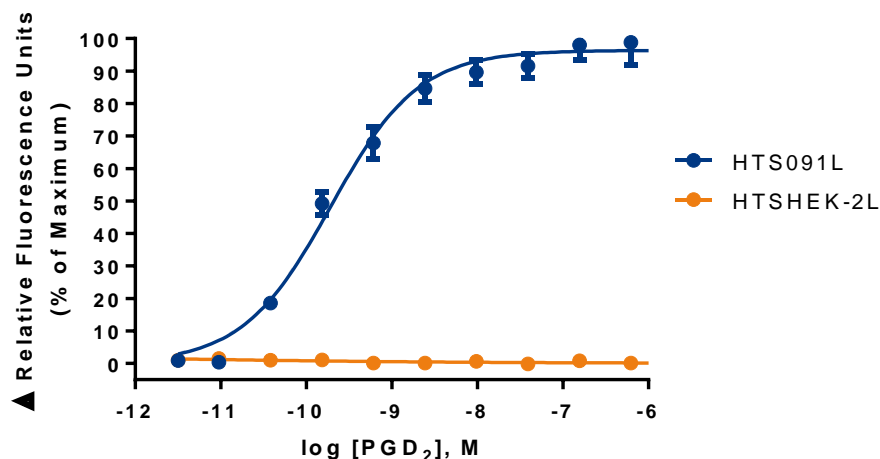


Figure 1. Representative data for activation of DP receptor stably expressed in HEK293 cells induced by PGD₂ using a fluorescent calcium flux assay. DP-expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand(s) with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPRTETRA® with ICCD camera. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

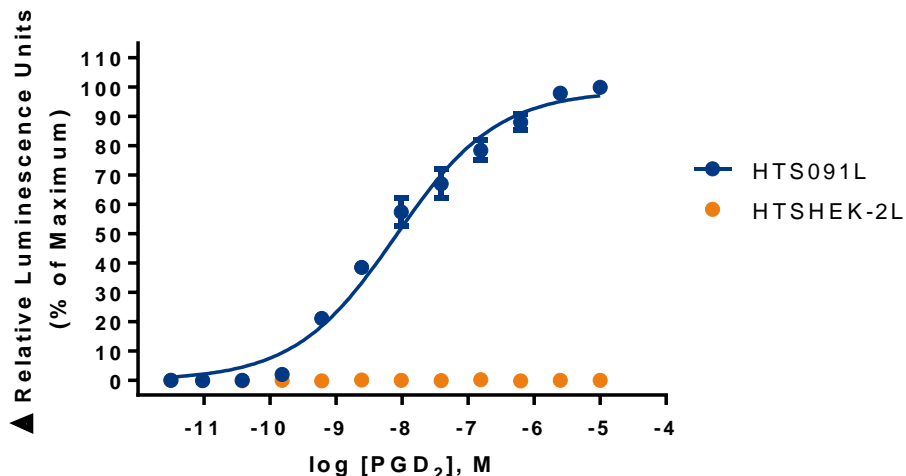


Figure 2. Representative data for activation of DP receptor stably expressed in HEK293 cells induced by PGD₂ using a luminescent calcium flux assay. DP-expressing HEK293 cells were loaded with 10 μ M coelenterazine; calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPRTETRA® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Similarly parental cells (catalog #: HTSHEK-2L) were tested to determine the specificity of the resulting signal.

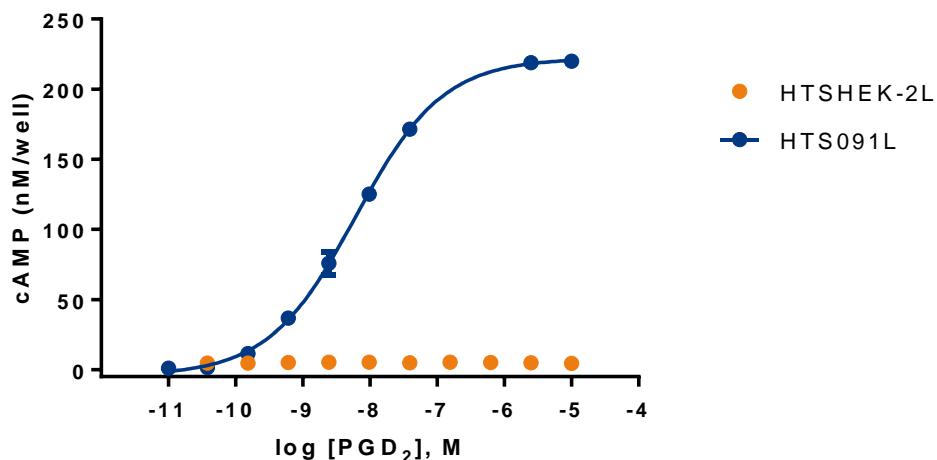


Figure 3. Representative data for activation of DP receptor stably expressed in HEK293 cells induced by Prostaglandin D2 using a cAMP accumulation assay. DP-expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate and the following day the cells were treated with ligand for 10 minutes in the presence of 100 μ M 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. EC₅₀ values of DP-expressing HEK293 cells.

| LIGAND | ASSAY | POTENCY EC ₅₀ (nM) | REFERENCE |
|------------------|-----------------------------|-------------------------------|------------------------|
| Prostaglandin D2 | Calcium Flux - Luminescence | 8.1* | Eurofins Internal Data |
| Prostaglandin D2 | Calcium Flux - Fluorescence | 1.0 | Eurofins Internal Data |
| Prostaglandin D2 | cAMP accumulation | 6.1 | 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 fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

| Description | Component | Concentration | Supplier and Product Number |
|-------------------------|----------------------------------|----------------|-----------------------------|
| Basal Medium | DMEM/F12 Medium | - | Gibco: 10565018 |
| | Fetal Bovine Serum (FBS) | 10% | Gibco: 1600044 |
| | Non-Essential Amino Acids (NEAA) | 1X | Hyclone: SH30238.01 |
| Selection Medium | Basal Medium (see above) | - | |
| | Geneticin (G418) | 400 μ g/ml | Gibco:10131-027 |
| | Puromycin | 1 μ g/ml | Gibco: A1113803 |
| | Hygromycin | 200 μ g/ml | Invitrogen: 10687010 |
| Dissociation | Sterile PBS | - | Hyclone: SH30028.03 |
| | 0.25% Trypsin-EDTA | - | Hyclone: SH30042.01 |
| CryoMedium | Basal Medium (see above) | 40% | |
| | Fetal Bovine Serum (FBS) | 50% | Gibco: 1600044 |
| | 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 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C 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, 37°C, 5% CO₂.
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 37° 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 |

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 |

Fluorescence

Table 5. 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 μ l (25 μ l for 384-well) |
| Dispense Height | 95 μ l (50 μ l for 384-well) |
| Dispense Speed | 50 μ l/sec |
| Expel Volume | 0 μ l |
| Analysis | Subtract Bias Sample 1 |

Table 6. Assay Materials (Not provided)

| Description | Supplier and Product Number |
|--|-----------------------------|
| HBSS | Invitrogen: 14025 |
| HEPES 1M Stock | EMD Millipore: TMS-003-C |
| Probenicid | Sigma: P8761 |
| Quest Fluo-8™, AM | AAT Bioquest: 21080 |
| PGD2 ligand | Cayman: 12010 |
| Non-Binding 96/384 well Plates (for ligand prep) | Corning: 3605/ 3574 |
| Black (clear Bottom) cell assay plates | Corning: 3904/ 3712 |
| Coelenterazine-h (250 μ g). Prepare to 10mM | Promega: S2011 |

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 |
| PGD2 ligand | Cayman: 12010 |
| cAMP Hi Range Kit | CisBio # 62AM6PEC |

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 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 μ L/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 37°C 5% CO₂ incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, pH 7.4) and Loading buffer (Assay buffer with 10 μ M coelenterazine). *Note: Please prepare coelenterazine stock according to Manufacturer's Recommendations at 10mM to allow for 1:1000 dilution into Loading buffer (10 μ M 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 μ L/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.

Assay Protocol – Fluorescence

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 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 μ L/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 37°C 5% CO₂ incubator for 18-24 h.
6. 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*
7. Remove medium from assay plate by quickly inverting plate on an absorbent pad and blotting to remove all Media Component.
8. Add Loading buffer to assay plate (100 μ L/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. 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. 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 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into appropriate assay microplate (100 μ L/well for 96-well plate, 25 μ L/well for 384-well plate).
5. When seeding is complete, place the assay plate at room temperature for 30 minutes.
6. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
7. Prepare Assay Buffer (HBSS, 20mM HEPES, pH 7.4)
8. Prepare anti-cAMP-Cryptate and cAMP-D2 conjugate stocks. Prepare stocks according to the Manufacturers Recommendations. Mix gently. Store at 10 to 35 °C after reconstitution. Reagent can only go through one cycle of freeze and thaw.

9. Prepare 1M IBMX Buffer: add 450 μ L of DMSO to 1 vial of 100 mg IBMX powder.
10. 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 μ L of 1M IBMX Buffer to 4.990 mL of Assay Buffer. Mix by vortex. Place the 2 mM of IBMX Buffer into 37°C incubator for at least 10 minutes.
11. Prepare 25 μ L/well of compounds for testing.
12. 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. Add 25 μ L of the 2 mM IBMX Buffer to the plate with the seeded cells. Tap plate gently 3-4 times. Cover plate and incubate inside 37°C incubator, until ready for compound addition.
13. Add 25 μ L compounds, internal control and test sample dilutions to cell assay plate. Cover plate and incubate for 15 to 25 min at 20-25°C
14. Prepare fresh working dilutions of anti-cAMP-Cryptate and cAMP-D2 conjugate in Lysis Buffer according to the Manufacturers Recommendations. Protect from light. Do not vortex.
15. It is imperative that detection reagents are added to plate in the following order:
Add 25 μ L cAMP-D2 conjugate/lysis buffer solution to each well of cell assay plate.
Add 25 μ L anti-cAMP-Cryptate/lysis buffer solution to each well of cell assay plate.
16. 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.
17. Read plate on a plate reader with 330 nm (excitation) and 665/620 nm (emission).
18. Calculate Ratio Emission 665/620 nm.

HOST CELL

HEK293

EXOGENOUS GENE EXPRESSION

Human PTGDR cDNA (Accession Number: NM_000953) and a proprietary mutant clytin photoprotein and promiscuous G protein expressed in a bicistronic vector

RELATED PRODUCTS

| Product Number | Description |
|----------------|--|
| HTSHEK-1L | ChemiBrite™ HEK stable cell line (control cells) |
| HTSHEK-2L | ChemiBrite™ HEK stable Gαqs cell line (control cells) |
| HTS185L | ChemiBrite™ HEK stable EP2 Prostanoid Receptor Cell Line |
| HTS092L | ChemiBrite™ HEK stable EP3 Prostanoid Receptor Cell Line |
| HTS142L | ChemiBrite™ HEK stable EP4 Prostanoid Receptor Cell Line |
| HTS081L | ChemiBrite™ HEK stable TP Prostanoid Receptor Cell Line |
| HTS093L | ChemiBrite™ HEK stable FP Prostanoid Receptor Cell Line |
| HTS091M | ChemiScreen™ DP Prostanoid receptor membrane prep |
| HTS091RTA | Ready-to-Assay™ DP Prostanoid receptor frozen cells |
| HTSCHEM-1RTA | Ready-to-Assay™ Chem-1 host frozen cells (control cells) |

REFERENCES

1. Matsuoka T. *et al.* (2000) Prostaglandin D₂ as a mediator of allergic asthma. *Science* 287: 2013-2017.
2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

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