eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ HEK293 Parental Frozen Cells with $G\alpha_{15}$

CATALOG NUMBER: HTSHEK-5LRTA

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.

Cloned HEK Parental Gα15 ChemiBrite cells were made by stable transfection of HEK293 cells with optimized quantities of plasmid encoding a novel variant of clytin and murine Gα15. These stability-tested cells are ideal for recombinant expression of target protein for use in calcium flux assays, for analysis of agonist, antagonist and modulator activity at the target protein, as well as cAMP assays.

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

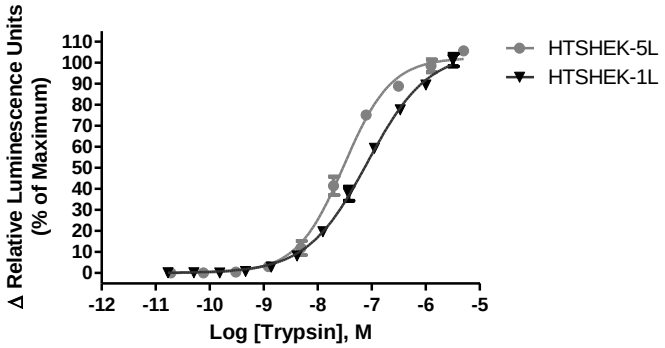

Figure 1. Representative data for luminescence response of clytin-HEK/Gα15 stable cell line compared with clytin-HEK cells (HTSHEK-1L). Calcium response was elicited by Trypsin activation of endogenous PAR receptors. Gα15–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature. Calcium flux response 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 600,000 RLU (Relative Light Units) as measured by AUC (area under curve) for 80 s post agonist addition using the provided protocol. Similarly parental cells (catalog #: HTSHEK-1L) were tested to Illustrate the clytin signal before addition of Gα15; the signal was 540,000 RLU.

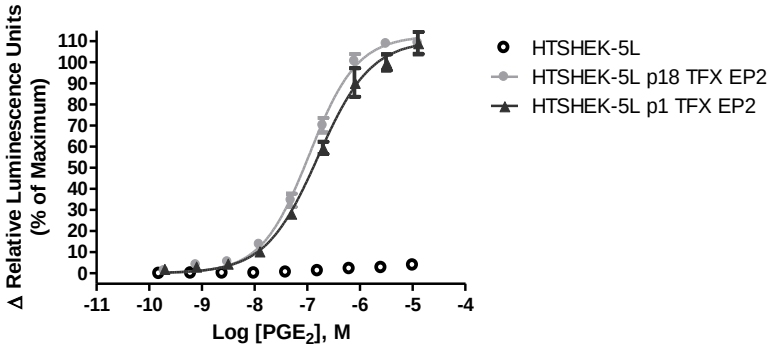

Figure 2.Representative luminescent calcium flux data illustrating recombinant expression of Prostanoid EP_2 receptor with stable HEKGα15/Clytin (HTSHEK-5L) cell line. Independent EP_2 receptor (TFX EP_2) transfections via electroporation were performed from continuous passage culture at passage 1 and 18 to illustrate stability of clytin and Gα15 expression. Cells were loaded with 10 µM coelenterazine for 3 h at room temperature. Calcium flux in response to PGE_2 was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format, with a final concentration of 0.5% DMSO. EC_{50} potency values of PGE_2 for EP_2 receptors expressed in HEK-5L were reported at 100.0 and 150.0 nM for passages 1 and 18, respectively.

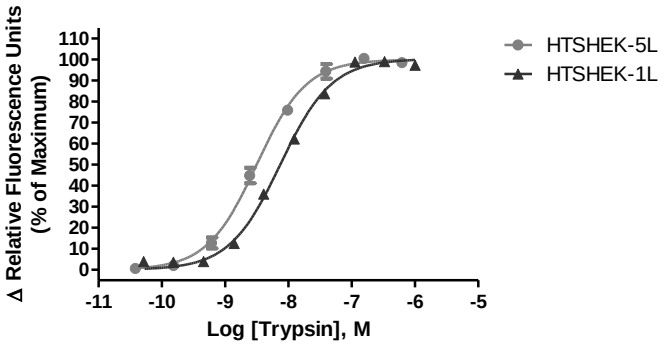

Figure 3. Representative data for fluorescence calcium response via activation of endogenous PAR receptor in clytin-HEK293/Gα15 stable cell line induced by Trypsin. Gα15–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with 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 9,000 RLU.

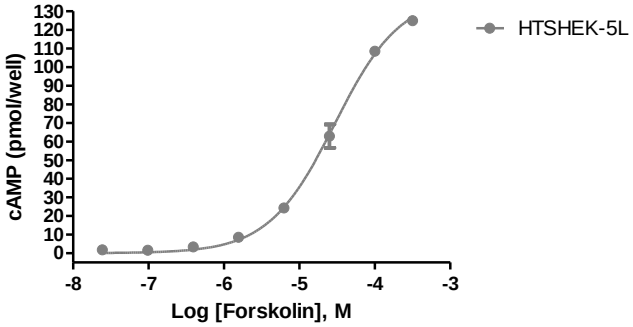

Figure 4. Representative data illustrating cAMP response in stable clytin-HEK293/Gα15 cells using a cAMP accumulation assay. Gα15–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with Forskolin for 10 minutes in the presence of 2.0 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.

Table 1. EC_{50} values of Gα15-expressing HEK293 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Trypsin	Calcium Flux - Luminescence	3.0	Eurofins Internal Data
Trypsin	Calcium Flux - Fluorescence	3.0	Eurofins Internal Data
Forskolin	cAMP accumulation	20000	Eurofins Internal Data

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)	-	
	Hygromycin	200 µg/ml	Invitrogen: 10687010
	Puromycin	1 µg/ml	Gibco: A1113803
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 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

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

Fluorescence

Table 3. 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 4. 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^{TM}, AM	AAT Bioquest: 21080
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

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.

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 $5x10^5$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, 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 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. 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.

HOST CELL

HEK293

EXONGENOUS GENE EXPRESSION

Murine Gα15 cDNA and a proprietary mutant clytin photoprotein, each expressed in a bicistronic vector

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-1L	ChemiBrite™ HEK293 Parental Stable Cell Line
HTSHEK-2L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqs
HTSHEK-3L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqi
HTSHEK-4L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqo
HTSHEK-5L	ChemiBrite™ HEK293 Parental Stable Cell Line with G α15
HTSHEK-6L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gα16

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