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PRODUCT DATASHEET

ChemiBrite™ HEK293 Parental Stable Cell Line with $G\alpha_{16}$

CATALOG NUMBER: HTSHEK-6L

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.

Cloned HEK Parental $G\alpha_{16}$ ChemiBrite cells were made by stable transfection of HEK293 cells with optimized quantities of plasmid encoding a novel variant of clytin and human $G\alpha_{16}$. 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

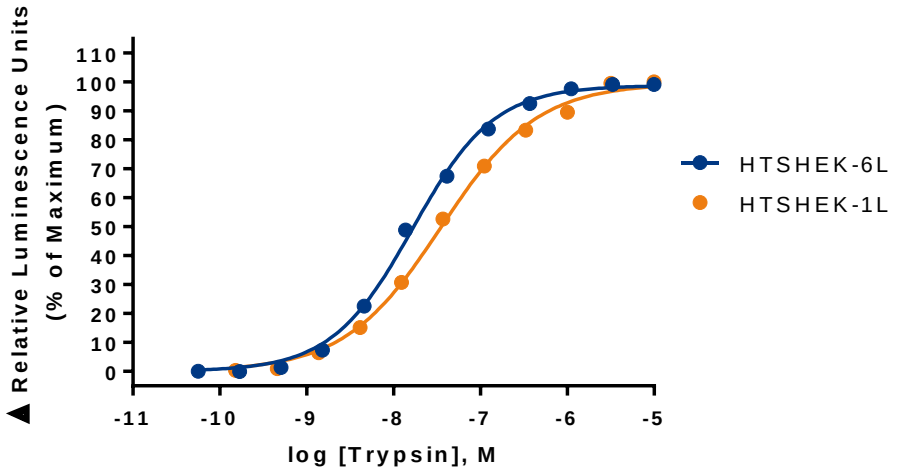

Figure 1. Representative data for luminescence response of clytin-HEK/Gα_{16} stable cell line compared with clytin-HEK cells (HTSHEK-1L). Calcium response was elicited by Trypsin activation of endogenous PAR receptors. Gα_{16} – expressing HEK293 cells were loaded with 10 µM coelenterazine. Calcium flux response 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-1L) were tested to Illustrate clytin stability.

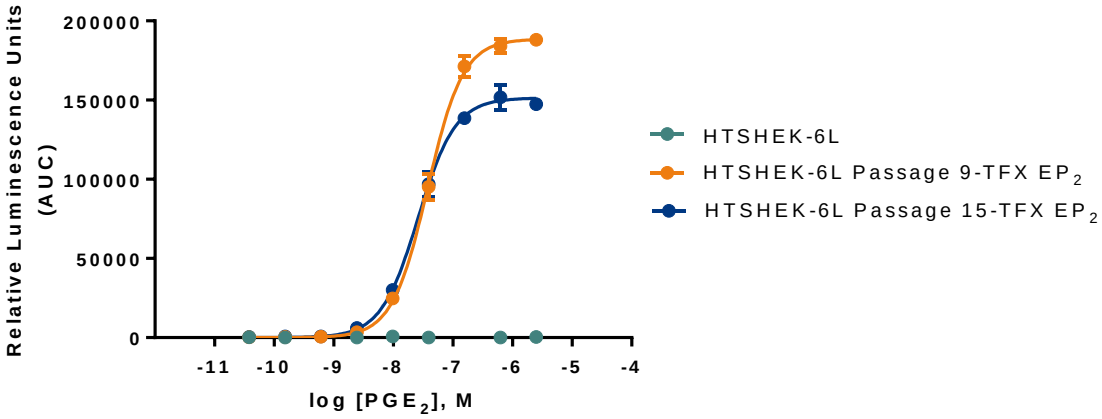

Figure 2.Representative luminescent calcium flux data illustrating recombinant expression of Prostanoid EP2 receptor with stable HEK/Gα_{16}/Clytin (HTSHEK-6L) cell line. Independent EP2 receptor transfections (TFX EP2) via electroporation were performed from continuous passage culture at passage 9 and 15 to illustrate stability of clytin and Gα16 expression. Cells were loaded with 10 µM coelenterazine. Calcium flux in response to PGE2 was determined on a Molecular Devices FLIPRTETRA® with ICCD camera in 96-well format, with a final concentration of 0.5% DMSO. EC50 potency values of PGE2 for EP2 receptors expressed in HTSHEK-6L were 37 and 26 nM for passages 9 and 15, respectively.

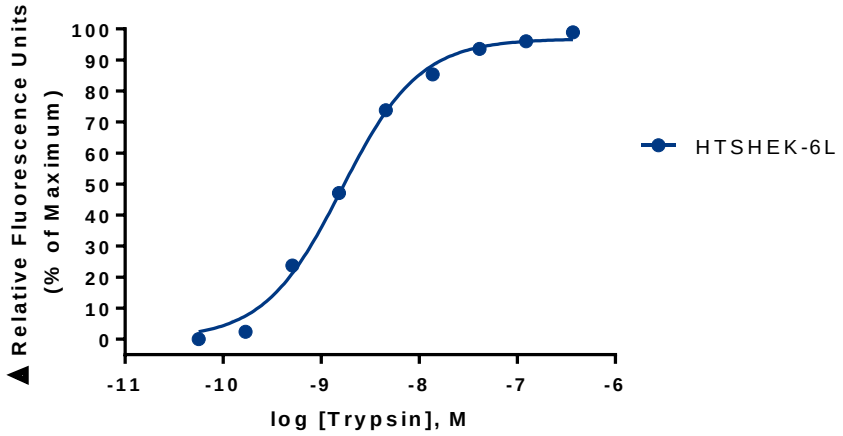

Figure 3. Representative data for fluorescence calcium response via activation of endogenous PAR receptor in clytin-HEK293/Gα_{16} stable cell line induced by Trypsin. Gα_{16}–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded 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.

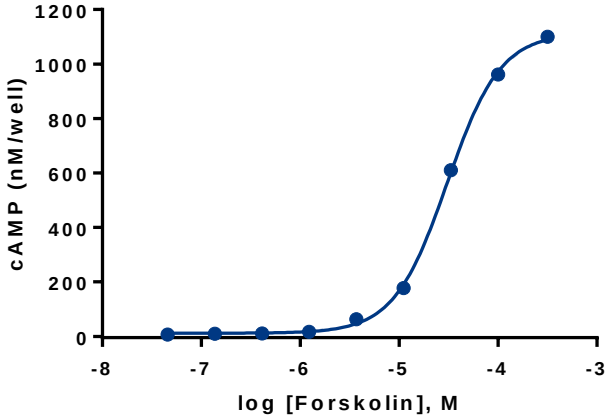

Figure 4. Representative data illustrating cAMP response in stable clytin-HEK293/Gα16 cells using a cAMP accumulation assay. Gα16–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with Forskolin for 10 minutes in the presence of 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.

Table 1. EC_{50} values of Gα_{16}-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Trypsin	Calcium Flux - Luminescence	16*	Eurofins Internal Data
Trypsin	Calcium Flux - Fluorescence	1.6	Eurofins Internal Data
Forskolin	cAMP accumulation	7000	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.

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 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 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 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
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

cAMP

Table 7. Settings for Plate Reader

Option	Setting
Excitation	300 nm
Emission	665/620 nm

Table 8. 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
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 $5x10^5$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 by quickly inverting plate on an absorbent pad and blotting to remove all Media Component.
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.

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 $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4.	Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/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 37ºC 5% CO_2 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 µL 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 µL 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 25uL/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 µL 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 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
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 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.
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 Gα16 cDNA and a proprietary mutant clytin photoprotein, each expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTSHEK-2L	ChemiBrite™ HEK293 Parental stable cell line with $G\alpha_{qs}$
HTSHEK-3L	ChemiBrite™ HEK293 Parental stable cell line with $G\alpha_{qi}$
HTSHEK-4L	ChemiBrite™ HEK293 Parental stable cell line with $G\alpha_{qo}$
HTSHEK-5L	ChemiBrite™ HEK293 Parental stable cell line with G_{15}
HTSHEK-6L	ChemiBrite™ HEK293 Parental stable cell line with G_{16}
HTSU2OS-1L	ChemiBrite™ U20S Parental stable cell line
HTSCHO-1L	ChemiBrite™ CHO Parental stable cell line

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