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PRODUCT DATASHEET

ChemiBrite™ GPBA Bile Acid Receptor Stable Cell Line

CATALOG NUMBER: HTS238L

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.

The bile acid lithocolic acid acts as an agonist on the GPBA GPCR, also known as GPR131 or TGR5, to increase intracellular cAMP in cells that express the receptor (Maruyama et al., 2002). Bile acids and other GPBA ligands increase glucagon-like peptide-1 (GLP-1) secretion from intestinal cells. Although Type II diabetics have impaired ability to secrete GLP-1, their responsiveness to this compound, which enhances the effects of insulin, is normal. Thus GPBA agonists are of interest in developing therapeutics for diabetes. Interestingly, it has been suggested that the anti-diabetic effect of olive leaves could in part be mediated by agonist activity at GPBA receptors by oleanolic acid (Sato et al. 2007). Eurofins' cloned human GPBA receptor-expressing ChemiBrite cells are made by stable transfection of HEK293 cells with ChemiBrite clytin, receptor and clytin 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 GPBA 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 Assays: Luminescent Mode and Fluorescent Mode ; cAMP accumulation

APPLICATION DATA

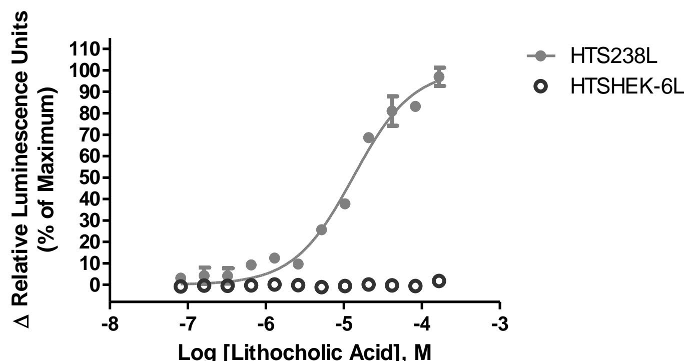

Figure 1. Representative data for activation of GPBA receptor stably expressed in HEK293 cells induced by Lithocholic Acid using a luminescent calcium flux assay. GPBA–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 hrs at room temperature and calcium flux in response to the indicated 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 1,000 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-6L) were tested to determine the specificity of the resulting signal.

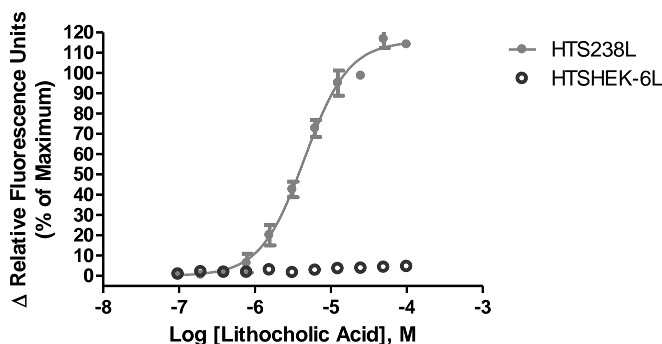

Figure 2. Representative data for activation of GPBA receptor stably expressed in HEK293 cells induced by Lithocholic Acid using a fluorescent calcium flux assay. GPBA–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, the following day the cells were loaded with a calcium 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 FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 5,000 RLU. Similarly parental cells (catalog #: HTSHEK-6L) were tested to determine the specificity of the resulting signal.

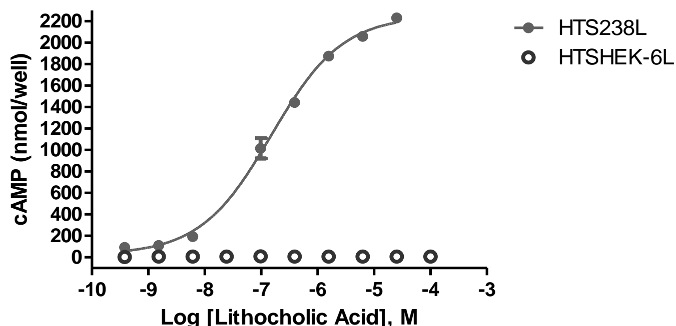

Figure 3. Representative data for activation of GPBA receptor stably expressed in HEK293 cells induced by Lithocholic Acid using a cAMP accumulation assay. GPBA–expressing HEK293 cells were seeded at 25,000 cells per well into a 96-well plate, and the following day the cells were treated with Ligand for 10 minutes in the presence of 100 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. Similarly parental cells (catalog #: HTSHEK-6L) were tested to determine the specificity of the resulting signal.

Table 1. EC50 values of LH-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Lithocholic Acid	Calcium Flux - Luminescence	15*	Eurofins Internal Data
Lithocholic Acid	Calcium Flux - Fluorescence	6	Eurofins Internal Data
Lithocholic Acid	cAMP accumulation	0.15	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 luminescence. The Z' value was 0.5.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12	-	Millipore: DF-041-B
	Fetal Bovine Serum (FBS)	10%	Gibco: 16000
	Non-Essential Amino Acids (NEAA)	1X	Millipore: TMS-001-C
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 ug/ml	EMD: 400053
	Geneticin (G418)	400 µg/ml	Invivogen: ant-gn-5
	Hygromycin	200 µg/ml	Invivogen: ant-hg-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.05% Trypsin-EDTA	-	Millipore: SM-2002-B
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Gibco: 16000
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

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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

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Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
BSA (Protease Free). Prepare to 1% in H_2O, filter	EMD: 126609
Lithocholic Acid ligand	Sigma: L6250
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.

HOST CELL

HEK293

EXOGENOUS GENE EXPRESSION

GPBAR1 cDNA (Accession Number: NM_170699; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein, and promiscuous G protein each expressed in a bicistronic vector

CODING SEQUENCE

```
ATG ACG CCC AAC AGC ACT GGC GAG GTG CCC AGC CCC ATT CCC AAG GGG GCT TTG
 M   T   P   N   S   T   G   E   V   P   S   P   I   P   K   G   A   L

GGG CTC TCC CTG GCC CTG GCA AGC CTC ATC ATC ACC GCG AAC CTG CTC CTA GCC
 G   L   S   L   A   L   A   S   L   I   I   T   A   N   L   L   L   A

CTG GGC ATC GCC TGG GAC CGC CGC CTG CGC AGC CCA CCT GCT GGC TGC TTC TTC
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 L   G   I   A   W   D   R   R   L   R   S   P   P   A   G   C   F   F

CTG AGC CTA CTG CTG GCT GGG CTG CTC ACG GGT CTG GCA TTG CCC ACA TTG CCA
 L   S   L   L   L   A   G   L   L   T   G   L   A   L   P   T   L   P

GGG CTG TGG AAC CAG AGT CGC CGG GGT TAC TGG TCC TGC CTC CTC GTC TAC TTG
 G   L   W   N   Q   S   R   R   G   Y   W   S   C   L   L   V   Y   L

GCT CCC AAC TTC TCC TTC CTC TCC CTG CTT GCC AAC CTC TTG CTG GTG CAC GGG
 A   P   N   F   S   F   L   S   L   L   A   N   L   L   L   V   H   G

GAG CGC TAC ATG GCA GTC CTG AGG CCA CTC CAG CCC CCT GGG AGC ATT CGG CTG
 E   R   Y   M   A   V   L   R   P   L   Q   P   P   G   S   I   R   L

GCC CTG CTC CTC ACC TGG GCT GGT CCC CTG CTC TTT GCC AGT CTG CCC GCT CTG
 A   L   L   L   T   W   A   G   P   L   L   F   A   S   L   P   A   L

GGG TGG AAC CAC TGG ACC CCT GGT GCC AAC TGC AGC TCC CAG GCT ATC TTC CCA
 G   W   N   H   W   T   P   G   A   N   C   S   S   Q   A   I   F   P

GCC CCC TAC CTG TAC CTC GAA GTC TAT GGG CTC CTG CTG CCC GCC GTG GGT GCT
 A   P   Y   L   Y   L   E   V   Y   G   L   L   L   P   A   V   G   A

GCT GCC TTC CTC TCT GTC CGC GTG CTG GCC ACT GCC CAC CGC CAG CTG CAG GAC
 A   A   F   L   S   V   R   V   L   A   T   A   H   R   Q   L   Q   D

ATC TGC CGG CTG GAG CGG GCA GTG TGC CGC GAT GAG CCC TCC GCC CTG GCC CGG
 I   C   R   L   E   R   A   V   C   R   D   E   P   S   A   L   A   R

GCC CTT ACC TGG AGG CAG GCA AGG GCA CAG GCT GGA GCC ATG CTG CTC TTC GGG
 A   L   T   W   R   Q   A   R   A   Q   A   G   A   M   L   L   F   G

CTG TGC TGG GGG CCC TAC GTG GCC ACA CTG CTC CTC TCA GTC CTG GCC TAT GAG
 L   C   W   G   P   Y   V   A   T   L   L   L   S   V   L   A   Y   E

CAG CGC CCG CCA CTG GGG CCT GGG ACA CTG TTG TCC CTC CTC TCC CTA GGA AGT
 Q   R   P   P   L   G   P   G   T   L   L   S   L   L   S   L   G   S

GCC AGT GCA GCG GCA GTG CCC GTA GCC ATG GGG CTG GGC GAT CAG CGC TAC ACA
 A   S   A   A   A   V   P   V   A   M   G   L   G   D   Q   R   Y   T

GCC CCC TGG AGG GCA GCC GCC CAA AGG TGC CTG CAG GGG CTG TGG GGA AGA GCC
 A   P   W   R   A   A   A   Q   R   C   L   Q   G   L   W   G   R   A

TCC CGG GAC AGT CCC GGC CCC AGC ATT GCC TAC CAC CCA AGC AGC CAA AGC AGT
 S   R   D   S   P   G   P   S   I   A   Y   H   P   S   S   Q   S   S


GTC GAC CTG GAC TTG AAC TAA
 V   D   L   D   L   N  STP
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RELATED PRODUCTS

Product Number	Description
HTSHEK-6L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gα16

REFERENCES

1. Maruyama et al. (2002) Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* 298:714-719
2. Sato et al. (2007) Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea*. *Biochem Biophys Res Commun* 362:793-798

Gonadotropin from Human but not Equine, Rat and Ovine Species. *Mol. Endocrinology* 5 (6)

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