eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ GPBA Bile Acid Receptor Frozen Cells

CATALOG NUMBER: HTS238LRTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

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 over night recovery, assays for calcium response.

ChemiBrite cells co-express a GPCR along with 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 mitochondrially expressed photoproteins. Luminescent calcium assays offer several advantages over fluorescent calcium assays including; lower substrate cost, 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). Cloned GPBA receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin and the receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. The cells have been cryopreserved at an optimal time post-transfection. Upon thaw, recovery, and loading, the cells are ready for luminescent, fluorescent and cAMP accumulation analysis of agonists, antagonists and modulators at the GPBA 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.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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Template version: 5. Modified Date: 27AUG14. Modified by: SGJ Steven Jarvis

APPLICATIONS

Calcium Flux Assays: Luminescent Mode and Fluorescent Mode; cAMP accumulation

APPLICATION DATA

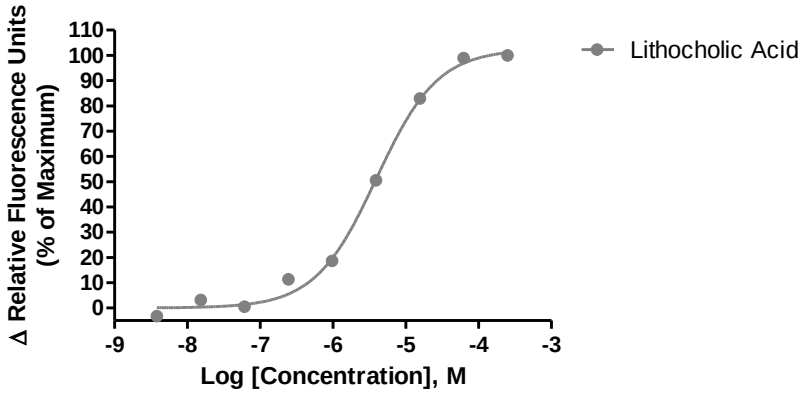

Figure 1. Representative data for activation of GPBA receptor. Calcium flux in GPBA–expressing HEK293 cell line induced by Lithocholic Acid. GPBA–expressing HEK293 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in triplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 8,000 RLU (Relative Light Units).

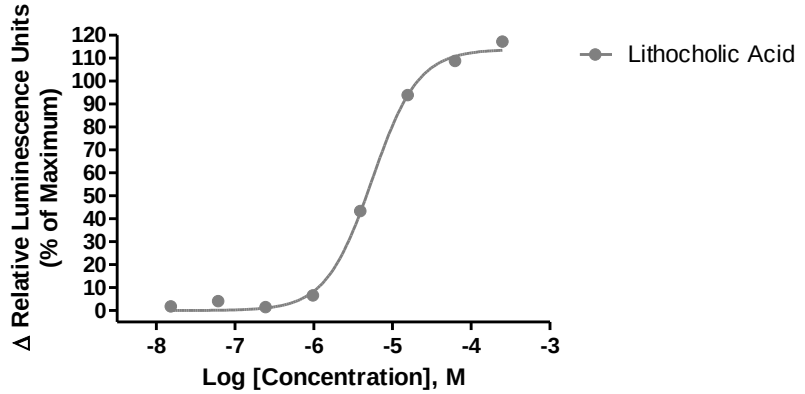

Figure 2. Representative data for activation of GPBA receptor expressed in HEK293 cells induced by Lithocholic Acid using a luminescent calcium flux assay. GPBA–expressing HEK293 cells were loaded with 10µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 35,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

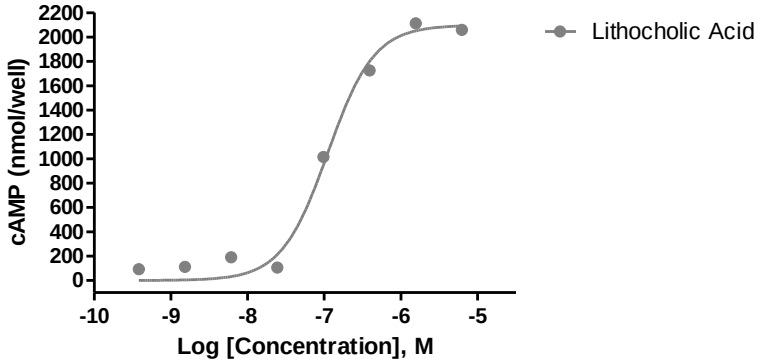

Figure 3. Representative data for activation of GPBA receptor stably expressed in HEK293 cells induced by Lithocholic Acid using a cAMP accumulation assay. GPBA–expressing HEK293 cells were seeded into a 96-well plate, and the following day the cells were treated with Lithocholic Acid for 15 minutes in the presence of 100µM IBMX to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy.

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

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
Lithocholic Acid	Calcium Flux - Fluorescence	3.9	Eurofins Internal Data
Lithocholic Acid	Calcium Flux - Luminescence	5.5	Eurofins Internal Data
Lithocholic Acid	cAMP accumulation	0.11	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

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
Lithocholic Acid ligand	Sigma: T7515
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

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

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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
 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
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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
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-6L	ChemiBrite™ HEK Parental cell line with Gα16 (control cells)

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

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