

PRODUCT DATASHEET

ChemiBrite™ FFA1 / GPR40 Free Fatty Acid Receptor Stable Cell Line

CATALOG NUMBER: HTS038L

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.

FFA1/GPR40 is a G_i/G_q-coupled GPCR that is activated by free medium and long-chain fatty acids. FFA1 receptors are expressed in brain tissue and insulin-producing β Pancreatic islets (Briscoe *et al.*, 2003). FFA1 receptors regulate insulin release from islets in response to long-chain fatty acids in the plasma (reviewed in Brown *et al.*, 2005). Eurofins cloned human FFA1 receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin and the FFA1 receptor. These stability-tested cells are ready for luminescent and fluorescent analysis of agonists, antagonists and modulators at the FFA1 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

APPLICATION DATA

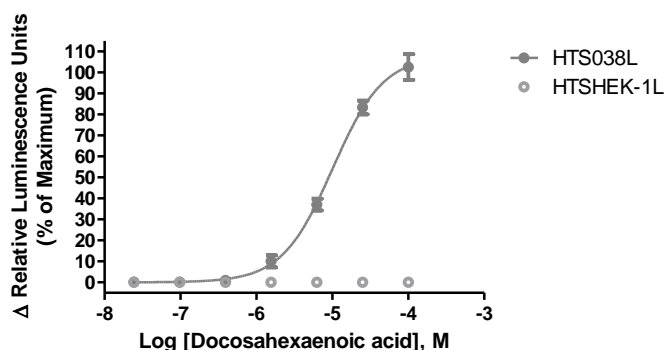


Figure 1. Representative data for activation of FFA1 receptor stably expressed in HEK293 cells induced by docosahexaenoic acid using a luminescent calcium flux assay. FFA1-expressing HEK293 cells were loaded with 10 μ M coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 300,000 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 determine the specificity of the resulting signal.

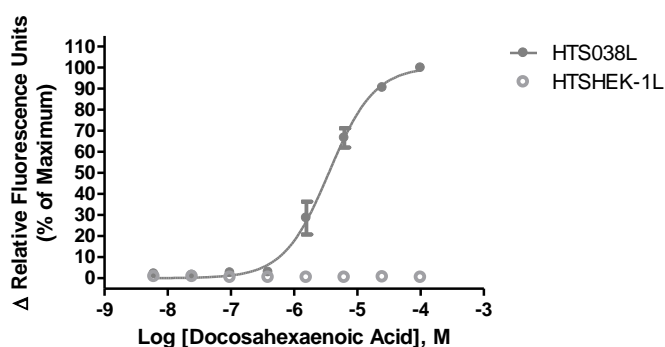


Figure 2. Representative data for activation of FFA1 receptor stably expressed in HEK293 cells induced by docosahexaenoic acid using a fluorescent calcium flux assay. FFA1-expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with 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 18,000 RLU. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

Table I. EC₅₀ values of FFA1-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
Docosahexaenoic acid	Calcium Flux - Luminescence	10000*	Eurofins Internal Data
Docosahexaenoic acid	Calcium Flux - Fluorescence	3000	Eurofins Internal Data
GW9508	Calcium Flux - Luminescence	10	Eurofins Internal Data
GW9508	Calcium Flux - Fluorescence	5	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, as defined with response to 500 uM docosahexaenoic acid is 0.6

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12 medium	-	Hyclone: SH30023
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH3007003
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH3023801
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	400 µg/ml	Invivogen: ant-gn-5
	Puromycin	1 µg/ml	Invivogen: ant-pr-1
Dissociation	Sterile PBS	-	Hyclone: SH3025601
	0.25% Trypsin-EDTA	-	Hyclone: SH3004201
CryoMedium	Basal Medium (see above)	40%	-
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH3007003
	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 2 min, RT, until cells dislodge. *If cells do not round up, place in 37° C incubator for 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	1.5	72
T150	30	4.0	24
T150	30	3.0	48
T150	30	2.0	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 TM , AM	AAT Bioquest: 21080
Docosahexaenoic acid ligand	Tocris:3687
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 5×10^5 cells/ml (i.e, if collected $5e6$ TC, $\frac{5e6}{5e5/ml} = 10$ 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^{TEIIRA}® 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 $5e6$ TC, $\frac{5e6}{5e5/ml} = 10$ 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 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. 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

EXOGENOUS GENE EXPRESSION

Human FFAR1 cDNA (Accession Number: NM_005303; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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ATG GAC CTG CCC CCG CAG CTC TCC TTC GGC CTC TAT GTG GCC GCC TTT GCG CTG GGC TTC CCG CTC
M D L P P Q L S F G L Y V A A F A L G F P L

AAC GTC CTG GCC ATC CGA GGC GCG ACG GCC CAC GCC CGG CTC CGT CTC ACC CCT AGC CTG GTC TAC
N V L A I R G A T A H A R L R L T P S L V Y

GCC CTG AAC CTG GGC TGC TCC GAC CTG CTG CTG ACA GTC TCT CTG CCC CTG AAG GCG GTG GAG GCG
A L N L G C S D L L L T V S L P L K A V E A

CTA GCC TCC GGG GCC TGG CCT CTG CCG GCC TCG CTG TGC CCC GTC TTC GCG GTG GCC CAC TTC TTC
L A S G A W P L P A S L C P V F A V A H F F

CCA CTC TAT GCC GGC GGG GGC TTC CTG GCC GCC CTG AGT GCA GGC CGC TAC CTG GGA GCA GCC TTC
P L Y A G G G F L A A L S A G R Y L G A A F

CCC TTG GGC TAC CAA GCC TTC CGG AGG CCG TGC TAT TCC TGG GGG GTG TGC GCG GCC ATC TGG GCC
P L G Y Q A F R R P C Y S W G V C A A I W A

CTC GTC CTG TGT CAC CTG GGT CTG GTC TTT GGG TTG GAG GCT CCA GGA GGC TGG CTG GAC CAC AGC
L V L C H L G L V F G L E A P G G W L D H S

AAC ACC TCC CTG GGC ATC AAC ACA CCG GTC AAC GGC TCT CCG GTC TGC CTG GAG GCC TGG GAC CCG
N T S L G I N T P V N G S P V C L E A W D P

GCC TCT GCC GGC CCG GCC CGC TTC AGC CTC TCT CTC CTG CTC TTT TTT CTG CCC TTG GCC ATC ACA
A S A G P A R F S L S L L L F F L P L A I T

GCC TTC TGC TAC GTG GGC TGC CTC CGG GCA CTG GCC CGC TCC GGC CTG ACG CAC AGG CCG AAG CTG
A F C Y V G C L R A L A R S G L T H R R K L

CGG GCC GCC TGG GTG GCC GGC GGG GCC CTC CTC ACG CTG CTG CTC TGC GTA GGA CCC TAC AAC GCC
R A A W V A G G A L L T L L L C V G P Y N A

TCC AAC GTG GCC AGC TTC CTG TAC CCC AAT CTA GGA GGC TCC TGG CCG AAG CTG GGG CTC ATC ACG
S N V A S F L Y P N L G G S W R K L G L I T

GGT GCC TGG AGT GTG GTG CTT AAT CCG CTG GTG ACC GGT TAC TTG GGA AGG GGT CCT GGC CTG AAG
G A W S V V L N P L V T G Y L G R G P G L K

ACA GTG TGT GCG GCA AGA ACG CAA GGG GGC AAG TCC CAG AAG TAA
T V C A A R T Q G G K S Q K .

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RELATED PRODUCTS

Product Number	Description
HTSHEK-1L	ChemiBrite™ HEK293 Parental Stable Cell Line
HTS198L	ChemiBrite™ GPR119 Class A Orphan Receptor stable cell line
HTS225L	ChemiBrite™ GPR120 Free Fatty Acid Receptor stable cell line
HTS063C	ChemiScreen™ GPR43/ FFA2 Free Fatty Acid Receptor stable cell line
HTS135C	ChemiScreen™ GPR41 / FFA3 Free Fatty Acid Receptor stable cell line

REFERENCES

1. Brown, A.J. *et al.* (2005) A family of fatty acid binding receptors. *DNA Cell Biol.* 24: 54-61.
2. Briscoe, C. *et al.* (2003) The orphan G protein–coupled receptor GPR40 is activated by medium and long chain fatty acids. *JBC* 278(13): 11303-11311

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