eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ FFA1/GPR40 Free Fatty Acid Receptor Frozen Cells

CATALOG NUMBER: HTS038LRTA

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.

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. Eurofins Discovery Services' cloned FFA1 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 FFA1 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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APPLICATIONS

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

APPLICATION DATA

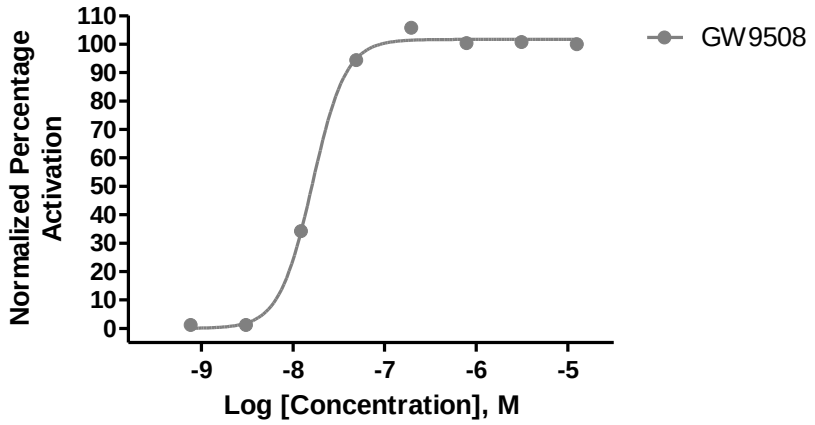

Figure 1. Representative data for activation of FFA1 receptor. Calcium flux in FFA1–expressing HEK293 cell line induced by GW9508. FFA1–expressing HEK293 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 34,000 RLU (Relative Light Units).

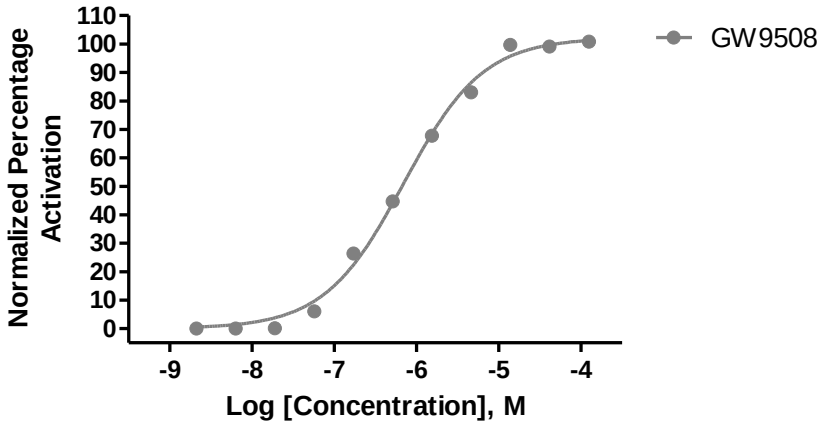

Figure 2. Representative data for activation of FFA1 receptor expressed in HEK293 cells induced by GW9508 using a luminescent calcium flux assay. FFA1–expressing HEK293 cells were loaded with 10µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 60,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

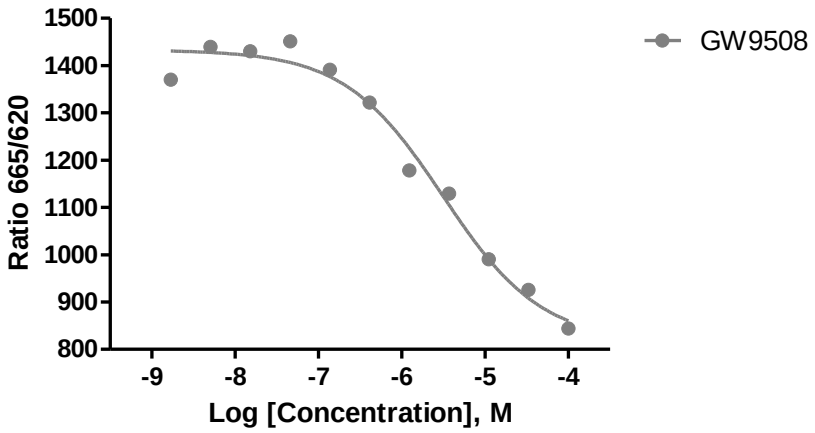

Figure 3. Representative data for activation of FFA1 receptor stably expressed in HEK293 cells induced by GW9508 using a cAMP accumulation assay. FFA1–expressing HEK293 cells were seeded into a 96-well plate, and the following day the cells were treated with GW9508 for 15 minutes in the presence of 100μM 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 FFA1-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
GW9508	Calcium Flux - Fluorescence	16	Eurofins Internal Data
GW9508	Calcium Flux - Luminescence	700	Eurofins Internal Data
GW9508	cAMP accumulation	3100	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™, AM	AAT Bioquest: 21080
GW9508 ligand	Tocris: 2649
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 5x10^5cells/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

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
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GCC TTC TGC TAC GTG GGC TGC CTC CGG GCA CTG GCC CGC TCC GGC CTG ACG CAC AGG CGG 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 CGG 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
HTS038L	ChemiBrite™ GPR40/FFA1 Free Fatty Acid Receptor stable cell line
HTS135RTA	Ready-to-Assay™ GPR41/FFA3 Free Fatty Acid Receptor frozen cells
HTS063RTA	Ready-to-Assay ™ GPR43/FFA2 Free Fatty Acid Receptor frozen cells
HTS135C	ChemiScreen™ GPR41/FFA3 Free Fatty Acid Receptor stable cell line
HTS063C	ChemiScreen™ GPR43/FFA2 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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