eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ GPR120 Receptor Frozen Cells

CATALOG NUMBER: HTS225LRTA

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.

Oh et al. (2010) demonstrated that Omega-3 fatty acid administration to obese mice inhibited inflammation and enhanced insulin sensitivity, but these effects were absent in GPR120 knockout mice. GPR120 agonists may prove to be useful in suppression of chronic inflammation seen in obesity, which could reduce insulin resistance and help restore glucose control. A recent review suggests that the effects of marine n-3 fatty acids on inflammatory markers studied in healthy subjects, those at high risk for developing Cardiovascular Disease, and those with diagnosed Cardiovascular Disease are as yet not conclusive (Myhrstad et al. 2011). Cloned GPR120 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 GPR120 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

APPLICATION DATA

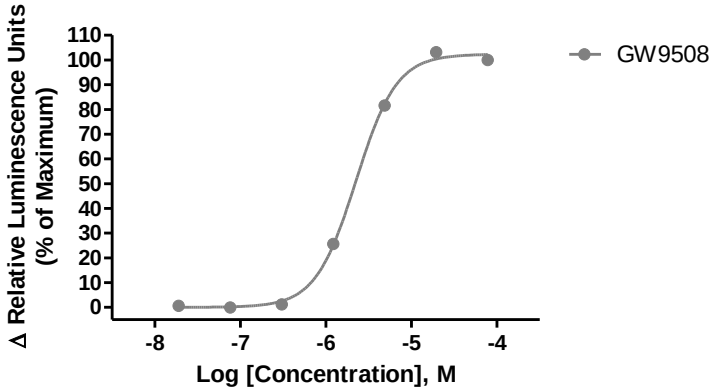

Figure 1. Representative data for activation of GPR120 receptor expressed in HEK293 cells induced by GW9508 using a luminescent calcium flux assay. GPR120–expressing HEK293 cells were loaded with 10µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 70,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

Table 1. Comparison of EC_{50} values of GPR120-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
GW9508	Calcium Flux - Luminescence	2.2	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

Table 3. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
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.

HOST CELL

HEK293

EXONGENOUS GENE EXPRESSION

Human GPR120 cDNA (Accession Number: BC101175.2; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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                                                ATG GTG AAC TTG AGG AAT GCG GTG CAT TCA TTC CTT
                                                 M   V   N   L   R   N   A   V   H   S   F   L

GTG CAC CTA ATT GGC CTA TTG GTT TGG CAA TGT GAT ATT TCT GTG AGC CCA GTA GCA GCT ATA GTA ACT
 V   H   L   I   G   L   L   V   W   Q   C   D   I   S   V   S   P   V   A   A   I   V   T

GAC ATT TTC AAT ACC TCC GAT GGT GGA CGC TTC AAA TTC CCA GAC GGG GTA CAA AAC TGG CCA GCA CTT
 D   I   F   N   T   S   D   G   G   R   F   K   F   P   D   G   V   Q   N   W   P   A   L

TCA ATC GTC ATC ATA ATA ATC ATG ACA ATA GGT GGC AAC ATC CTT GTG ATC ATG GCA GTA AGC ATG GAA
 S   I   V   I   I   I   I   M   T   I   G   G   N   I   L   V   I   M   A   V   S   M   E

AAG AAA CTG CAC AAT GCC ACC AAT TAC TTC TTA ATG TCC CTA GCC ATT GCT GAT ATG CTA GTG GGA CTA
 K   K   L   H   N   A   T   N   Y   F   L   M   S   L   A   I   A   D   M   L   V   G   L

CTT GTC ATG CCC CTG TCT CTC CTG GCA ATC CTT TAT GAT TAT GTC TGG CCA CTA CCT AGA TAT TTG TGC
 L   V   M   P   L   S   L   L   A   I   L   Y   D   Y   V   W   P   L   P   R   Y   L   C

CCC GTC TGG ATT TCT TTA GAT GTT TTA TTT TCA ACA GCG TCC ATC ATG CAC CTC TGC GCT ATA TCG CTG
 P   V   W   I   S   L   D   V   L   F   S   T   A   S   I   M   H   L   C   A   I   S   L

GAT CGG TAT GTA GCA ATA CGT AAT CCT ATT GAG CAT AGC CGT TTC AAT TCG CGG ACT AAG GCC ATC ATG
 D   R   Y   V   A   I   R   N   P   I   E   H   S   R   F   N   S   R   T   K   A   I   M

AAG ATT GCT ATT GTT TGG GCA ATT TCT ATA GGT GTA TCA GTT CCT ATC CCT GTG ATT GGA CTG AGG GAC
 K   I   A   I   V   W   A   I   S   I   G   V   S   V   P   I   P   V   I   G   L   R   D

GAA GAA AAG GTG TTC GTG AAC AAC ACG ACG TGC GTG CTC AAC GAC CCA AAT TTC GTT CTT ATT GGG TCC
 E   E   K   V   F   V   N   N   T   T   C   V   L   N   D   P   N   F   V   L   I   G   S

TTC GTA GCT TTC TTC ATA CCG CTG ACG ATT ATG GTG ATT ACG TAT TGC CTG ACC ATC TAC GTT CTG CGC
 F   V   A   F   F   I   P   L   T   I   M   V   I   T   Y   C   L   T   I   Y   V   L   R

CGA CAA GCT TTG ATG TTA CTG CAC GGC CAC ACC GAG GAA CCG CCT GGA CTA AGT CTG GAT TTC CTG AAG
 R   Q   A   L   M   L   L   H   G   H   T   E   E   P   P   G   L   S   L   D   F   L   K

TGC TGC AAG AGG AAT ACG GCC GAG GAA GAG AAC TCT GCA AAC CCT AAC CAA GAC CAG AAC GCA CGC CGA
 C   C   K   R   N   T   A   E   E   E   N   S   A   N   P   N   Q   D   Q   N   A   R   R

AGA AAG AAG AAG GAG AGA CGT CCT AGG GGC ACC ATG CAG GCT ATC AAC AAT GAA AGA AAA GCT TCG AAA
 R   K   K   K   E   R   R   P   R   G   T   M   Q   A   I   N   N   E   R   K   A   S   K

GTC CTT GGG ATT GTT TTC TTT GTG TTT CTG ATC ATG TGG TGC CCA TTT TTC ATT ACC AAT ATT CTG TCT
 V   L   G   I   V   F   F   V   F   L   I   M   W   C   P   F   F   I   T   N   I   L   S

GTT CTT TGT GAG AAG TCC TGT AAC CAA AAG CTC ATG GAA AAG CTT CTG AAT GTG TTT GTT TGG ATT GGC
 V   L   C   E   K   S   C   N   Q   K   L   M   E   K   L   L   N   V   F   V   W   I   G

TAT GTT TGT TCA GGA ATC AAT CCT CTG GTG TAT ACT CTG TTC AAC AAA ATT TAC CGA AGG GCA TTC TCC
 Y   V   C   S   G   I   N   P   L   V   Y   T   L   F   N   K   I   Y   R   R   A   F   S

AAC TAT TTG CGT TGC AAT TAT AAG GTA GAG AAA AAG CCT CCT GTC AGG CAG ATT CCA AGA GTT GCC GCC
 N   Y   L   R   C   N   Y   K   V   E   K   K   P   P   V   R   Q   I   P   R   V   A   A

ACT GCT TTG TCT GGG AGG GAG CTT AAT GTT AAC ATT TAT CGG CAT ACC AAT GAA CCG GTG ATC GAG AAA
 T   A   L   S   G   R   E   L   N   V   N   I   Y   R   H   T   N   E   P   V   I   E   K

GCC AGT GAC AAT GAG CCC GGT ATA GAG ATG CAA GTT GAG AAT TTA GAG TTA CCA GTA AAT CCC TCC AGT
 A   S   D   N   E   P   G   I   E   M   Q   V   E   N   L   E   L   P   V   N   P   S   S

GTG GTT AGC GAA AGG ATT ACG AGT GTG TGA
 V   V   S   E   R   I   T   S   V  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-1L	ChemiBrite™ HEK293 stable cell line (control cells)
HTS198LRTA	Ready-to-Assay™ GPR119 receptor frozen cells

REFERENCES

1. Oh et al. (2010) GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* 142(5):687-698.
2. Myhrstad et al. (2011) Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors. *Inflamm Res*. 60(4):309-319.
3. Hirasawa et al. (2005). Free fatty acids regulate gut incretion in glucagon-like peptide-1 secretion through GPR120. *Nature Medicine*:11:90-94
4. Briscoe et al. (2006) Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of the agonist and antagonist small molecules *Br J Pharm* 146:619-628

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