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PRODUCT DATASHEET

Ready-to-Assay™ FFA3/GPR41 Free Fatty Acid Receptor Frozen Cells

CATALOG NUMBER: HTS135RTA

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 overnight recovery, assays for calcium response.

GPR41 is a GPCR that, along with GPR43, is activated by short chain carboxylic acids formate, acetate, proprionate, butyrate and pentanoate (Brown *et al*., 2003; Brown *et al*., 2005). Binding of these ligands to GPR41 selectively activates Gi to inhibit cAMP accumulation. Expression of GPR41 is prominent in adipose tissue, increases during differentiation of cultured adipocytes, and allows short chain carboxylic acids to stimulate leptin synthesis in adipocytes (Xiong *et al*., 2003) Cloned human GPR41-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant GPR41 expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at GPR41 (FFA3).

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

APPLICATION DATA

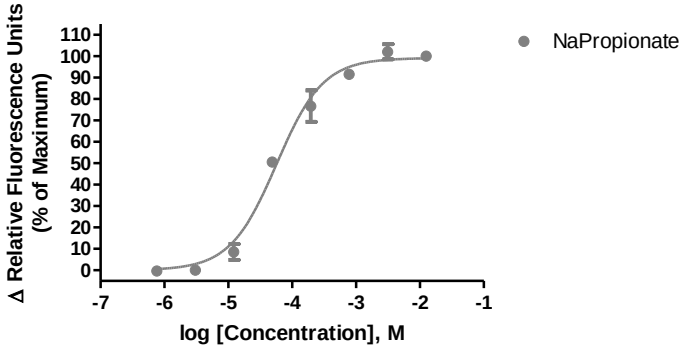

Figure 1. Representative data for activation of GPR41 receptor. Calcium flux in GPR41–expressing Chem-4 cell line induced by Sodium Propionate. GPR41–expressing Chem-4 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,000 RLU (Relative Light Units).

Table 1. EC_{50} value of GPR41-expressing Chem-4 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
NaPropionate	Calcium Flux	5.7	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
NaPriopionate ligand	Sigma: P1880
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

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	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

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HOST CELL

Chem-4, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein, as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

FFAR3 cDNA (Accession Number: NM_005304; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                    ATG GAT ACA GGC CCC GAC CAG TCC TAC TTC TCC GGC AAT CAC TGG TTC GTC TTC
                     M   D   T   G   P   D   Q   S   Y   F   S   G   N   H   W   F   V   F

TCG GTG TAC CTT CTC ACT TTC CTG GTG GGG CTC CCC CTC AAC CTG CTG GCC CTG GTG GTC TTC GTG GGC
 S   V   Y   L   L   T   F   L   V   G   L   P   L   N   L   L   A   L   V   V   F   V   G

AAG CTG CAG CGC CGC CCG GTG GCC GTG GAC GTG CTC CTG CTC AAC CTG ACC GCC TCG GAC CTG CTC CTG
 K   L   Q   R   R   P   V   A   V   D   V   L   L   L   N   L   T   A   S   D   L   L   L

CTG CTG TTC CTG CCT TTC CGC ATG GTG GAG GCA GCC AAT GGC ATG CAC TGG CCC CTG CCC TTC ATC CTC
 L   L   F   L   P   F   R   M   V   E   A   A   N   G   M   H   W   P   L   P   F   I   L

TGC CCA CTC TCT GGA TTC ATC TTC TTC ACC ACC ATC TAT CTC ACC GCC CTC TTC CTG GCA GCT GTG AGC
 C   P   L   S   G   F   I   F   F   T   T   I   Y   L   T   A   L   F   L   A   A   V   S

ATT GAA CGC TTC CTG AGT GTG GCC CAC CCA CTG TGG TAC AAG ACC CGG CCG AGG CTG GGG CAG GCA GGT
 I   E   R   F   L   S   V   A   H   P   L   W   Y   K   T   R   P   R   L   G   Q   A   G

CTG GTG AGT GTG GCC TGC TGG CTG TTG GCC TCT GCT CAC TGC AGC GTG GTC TAC GTC ATA GAA TTC TCA
 L   V   S   V   A   C   W   L   L   A   S   A   H   C   S   V   V   Y   V   I   E   F   S

GGG GAC ATC TCC CAC AGC CAG GGC ACC AAT GGG ACC TGC TAC CTG GAG TTC CGG AAG GAC CAG CTA GCC
 G   D   I   S   H   S   Q   G   T   N   G   T   C   Y   L   E   F   R   K   D   Q   L   A

ATC CTC CTG CCC GTG CGG CTG GAG ATG GCT GTG GTC CTC TTT GTG GTC CCG CTG ATC ATC ACC AGC TAC
 I   L   L   P   V   R   L   E   M   A   V   V   L   F   V   V   P   L   I   I   T   S   Y

TGC TAC AGC CGC CTG GTG TGG ATC CTC GGC AGA GGG GGC AGC CAC CGC CGG CAG AGG AGG GTG GCG GGG
 C   Y   S   R   L   V   W   I   L   G   R   G   G   S   H   R   R   Q   R   R   V   A   G

CTG TTG GCG GCC ACG CTG CTC AAC TTC CTT GTC TGC TTT GGG CCC TAC AAC GTG TCC CAT GTC GTG GGC
 L   L   A   A   T   L   L   N   F   L   V   C   F   G   P   Y   N   V   S   H   V   V   G

TAT ATC TGC GGT GAA AGC CCG GCG TGG AGG ATC TAC GTG ACG CTT CTC AGC ACC CTG AAC TCC TGT GTC
 Y   I   C   G   E   S   P   A   W   G   I   Y   V   T   L   L   S   T   L   N   S   C   V

GAC CCC TTT GTC TAC TAC TTC TCC TCC TCC GGG TTC CAA GCC GAC TTT CAT GAG CTG CTG AGG AGG TTG
 D   P   F   V   Y   Y   F   S   S   S   G   F   Q   A   D   F   H   E   L   L   R   R   L

TGT GGG CTC TGG GGC CAG TGG CAG CAG GAG AGC AGC ATG GAG CTG AAG GAG CAG AAG GGA GGG GAG GAG
 C   G   L   W   G   Q   W   Q   Q   E   S   S   M   E   L   K   E   Q   K   G   G   E   E

CAG AGA GCG GAC CGA CCA GCT GAA AGA AAG ACC AGT GAA CAC TCA CAG GGC TGT GGA ACT GGT GGC CAG
 Q   R   A   D   R   P   A   E   R   K   T   S   E   H   S   Q   G   C   G   T   G   G   Q

GTG GCC TGT GCT GAA AGC TGA
 V   A   C   A   E   S  Stp
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS135M	ChemiScreen™ FFA3/GPR41 Receptor membrane prep

* Note: Chem-4 cells are derived from Chem-1 cells

REFERENCES

1. Brown AJ *et al.* (2003)The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278: 11312-11319.
2. Brown AJ *et al.* (2005) A family of fatty acid binding receptors. *DNA Cell Biol.* 24: 54-61.

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