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PRODUCT DATASHEET

Ready-to-Assay™ FFA2 / GPR43 Free Fatty Acid Receptor Frozen Cells

CATALOG NUMBER: HTS063RTA

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.

FFA2, also known as GPR43, is a GPCR that, along with GPR41, is activated by short chain carboxylic acids formate, acetate, proprionate, butyrate and pentanoate. Neutrophils and other leukocytes selectively express FFA2, and FFA2 mediates calcium flux and chemotaxis in these cells by coupling to both G_q and G_i (Brown *et al*., 2003; Le Poul *et al*., 2003). Cloned human FFA2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant FFA2 expression on the cell surface for robust signaling via the calcium pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at FFA2.

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

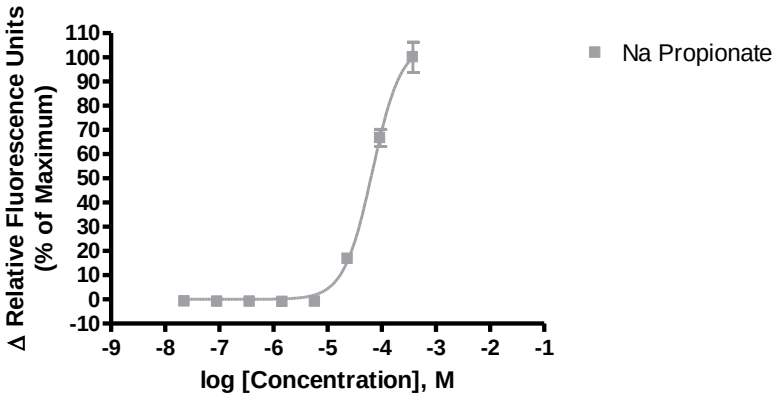

Figure 1. Representative data for activation of FFA2 receptor. Calcium flux in FFA2 –expressing Chem-1 cell line induced by sodium propionate. FFA2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 5,500 RLU (Relative Light Units).

Table 1. EC_{50} value of FFA2 -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
NaPropionate	Calcium Flux	100	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
Sodium propionate 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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

FFAR2 cDNA (Accession Number: BC096201; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG CTG CCG GAC TGG AAG AGC TCC TTG ATC CTC ATG GCT TAC ATC ATC ATC TTC CTC ACT GGC CTC CCT GCC AAC CTC CTG
GCC CTG CGG GCC TTT GTG GGG CGG ATC CGC CAG CCC CAG CCT GCA CCT GTG CAC ATC CTC CTG CTG AGC CTG ACG CTG GCC
GAC CTC CTC CTG CTG CTG CTG CTG CCC TTC AAG ATC ATC GAG GCT GCG TCG AAC TTC CGC TGG TAC CTG CCC AAG GTC GTC
TGC GCC CTC ACG AGT TTT GGC TTC TAC AGC AGC ATC TAC TGC AGC ACG TGG CTC CTG GCG GGC ATC AGC ATC GAG CGC TAC
CTG GGA GTG GCT TTC CCC GTG CAG TAC AAG CTC TCC CGC CGG CCT CTG TAT GGA GTG ATT GCA GCT CTG GTG GCC TGG GTT
ATG TCC TTT GGT CAC TGC ACC ATC GTG ATC ATC GTT CAA TAC TTG AAC ACG ACT GAG CAG GTC AGA AGT GGC AAT GAA ATT
ACC TGC TAC GAG AAC TTC ACC GAT AAC CAG TTG GAC GTG GTG CTG CCC GTG CGG CTG GAG CTG TGC CTG GTG CTC TTC TTC
ATC CCC ATG GCA GTC ACC ATC TTC TGC TAC TGG CGT TTT GTG TGG ATC ATG CTC TCC CAG CCC CTT GTG GGG GCC CAG AGG
CGG CGC CGA GCC GTG GGG CTG GCT GTG GTG ATG CTG CTC AAT TTC CTG GTG TGC TTC GGA CCT TAC AAC GTG TCC CAC CTG
GTG GGG TAT CAC CAG AGA AAA AGC CCC TGG TGG CGG TCA ATA GCC GTG GTG TTC AGT TCA CTC AAC GCC AGT CTG GAC CCC
CTG CTC TTC TAT TTC TCT TCT TCA GTG GTG CGC AGG GCA TTT GGG AGA GGG CTG CAG GTG CTG CGG AAT CAG GGC TCC TCC
CTG TTG GGA CGC AGA GGC AAA GAC ACA GCA GAG GGG ACA AAT GAG GAC AGG GGT GTG GGT CAA GGA GAA GGG ATG CCA AGT
TCG GAC TTC ACT ACA GAG TGA
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS063M	ChemiScreen™ FFA2 / GPR43 free fatty acid receptor membrane prep

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. Le Poul E *et al.* (2003) Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* 278: 25481-9.

User Agreement (Label License)

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