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PRODUCT DATASHEET

Ready-to-Assay™ FPRL1 N-formylpeptide Receptor Frozen Cells

CATALOG NUMBER: HTS056RTA

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.

FPRL1 (formyl peptide receptor-like 1, also known as FPR2) is a GPCR that belongs to the N-formyl peptide receptor family. Initially described as a receptor for lipoxin A4, FPRL1 has been shown to bind to a synthetic peptide WKYMVm, amyloid beta peptides, and N-formylated mitochondrial peptides and mediates phagocyte chemotaxis (Fiore *et al.*, 1994; Le *et al.*, 1999; Rabiet *et al.*, 2005; Iribarren *et al.*, 2005 Eurofins' cloned human FPRL1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant FPRL1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 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 FPRL1.

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.

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

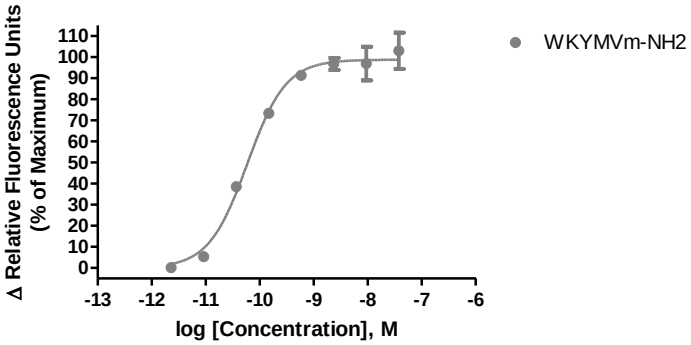

Figure 1. Representative data for activation of FPRL1 receptor. Calcium flux in FPRL1–expressing Chem-1 cell line induced by WKYMvm-NH2. FPRL1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 22,500 RLU (Relative Light Units).

Table 1. EC_{50} value of FPRL1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
WKYMvm-NH2	Calcium Flux	0.06	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: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
WKYMVm-NH2 ligand	Anaspec: 27069
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

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

FPR2 cDNA (Accession Number: NM_001462; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS160C	ChemiScreen™ FPRL1 N-formylpeptide receptor stable cell line
HTS160M	ChemiScreen™ FPRL1 N-formylpeptide receptor membrane prep

REFERENCES

1. Fiore S et al. (1994) Identification of a human cDNA encoding a functional high affinity lipoxin A receptor. *J. Exp. Med*. 180: 253-260.
2. Iribarren P et al. (2005) Role of formyl peptide receptor-like 1 (FPRL1/FPR2) in mononuclear phagocyte responses in Alzheimer disease. *Immunol. Res*. 31: 165-76.
3. Le Y et al. (1999) Utilization of two seven-transmembrane, G protein-coupled receptors, formyl peptide receptor-like 1 and formyl peptide recptor, byt the synthetic hexapeptide WKYMVm for human phagocyte activation. *J. Immunol*. 163: 6777-6784.
4. Rabiet MJ et al. (2005) Human mitochondria-derived N-formylated peptides are novel agonists equally active on FPR and FPRL1, while Listeria monocytogenes-derived peptides preferentially activate FPR. *Eur. J. Immunol*. 35: 2486-95.

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