eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ EP_4 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS142RTA

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.

Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and further modified by isomerases and synthases. Cells rapidly secrete prostanoids after synthesis, whereupon the prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostaglandin PGE_2 causes pain, vasodilation, immunosuppression of T cells, bone remodeling and promotion of carcinogenesis. Four related GPCRs, EP_1, EP_2, EP_3 and EP_4, each bind to PGE_2, but the different G protein coupling status of each receptor leads to distinct biological effects. EP_4 couples primarily to G_s to increase intracellular cAMP levels. During neonatal development, EP_4 participates in closure of the ductus arteriosus, a process required for switching circulation from the placenta to the lungs (Nguyen *et al*., 1997). In addition, EP_4 mediates PGE_2-induced bone formation by promoting osteoblastogenesis, and selective EP_4 agonists are being evaluated as potential treatments for osteoporosis (Yoshida *et al*., 2002). Cloned human EP_4-expressing cells are made in the Chem-1 host, which supports high levels of recombinant EP_4 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 and antagonists at EP_4

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

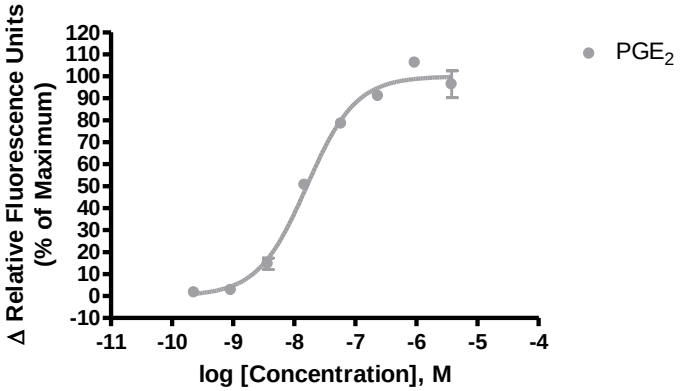

Figure 1. Representative data for activation of EP_4 receptor. Calcium flux in EP_4–expressing Chem-1 cell line induced by Prostagladin E2 (PDE_2). EP_4–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,500 RLU (Relative Light Units).

Table 1. EC_{50} values of EP_4-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
PDE2	Calcium Flux	16	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
PDE_2 ligand	Cayman: 14010
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

PTGER4 cDNA (Accession Number: NM_000958; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG TCC ACT CCC GGG GTC AAT TCG TCC GCC TCC TTG
                                                  M   S   T   P   G   V   N   S   S   A   S   L

AGC CCC GAC CGG CTG AAC AGC CCA GTG ACC ATC CCG GCG GTG ATG TTC ATC TTC GGG GTG GTG GGC AAC
 S   P   D   R   L   N   S   P   V   T   I   P   A   V   M   F   I   F   G   V   V   G   N

CTG GTG GCC ATC GTG GTG CTG TGC AAG TCG CGC AAG GAG CAG AAG GAG ACG ACC TTC TAC ACG CTG GTA
 L   V   A   I   V   V   L   C   K   S   R   K   E   Q   K   E   T   T   F   Y   T   L   V

TGT GGG CTG GCT GTC ACC GAC CTG TTG GGC ACT TTG TTG GTG AGC CCG GTG ACC ATC GCC ACG TAC ATG
 C   G   L   A   V   T   D   L   L   G   T   L   L   V   S   P   V   T   I   A   T   Y   M

AAG GGC CAA TGG CCC GGG GGC CAG CCG CTG TGC GAG TAC AGC ACC TTC ATT CTG CTC TTC TTC AGC CTG
 K   G   Q   W   P   G   G   Q   P   L   C   E   Y   S   T   F   I   L   L   F   F   S   L

TCC GGC CTC AGC ATC ATC TGC GCC ATG AGT GTC GAG CGC TAC CTG GCC ATC AAC CAT GCC TAT TTC TAC
 S   G   L   S   I   I   C   A   M   S   V   E   R   Y   L   A   I   N   H   A   Y   F   Y

AGC CAC TAC GTG GAC AAG CGA TTG GCG GGC CTC ACG CTC TTT GCA GTC TAT GCG TCC AAC GTG CTC TTT
 S   H   Y   V   D   K   R   L   A   G   L   T   L   F   A   V   Y   A   S   N   V   L   F

TGC GCG CTG CCC AAC ATG GGT CTC GGT AGC TCG CGG CTG CAG TAC CCA GAC ACC TGG TGC TTC ATC GAC
 C   A   L   P   N   M   G   L   G   S   S   R   L   Q   Y   P   D   T   W   C   F   I   D

TGG ACC ACC AAC GTG ACG GCG CAC GCC GCC TAC TCC TAC ATG TAC GCG GGC TTC AGC TCC TTC CTC ATT
 W   T   T   N   V   T   A   H   A   A   Y   S   Y   M   Y   A   G   F   S   S   F   L   I

CTC GCC ACC GTC CTC TGC AAC GTG CTT GTG TGC GGC GCG CTG CTC CGC ATG CAC CGC CAG TTC ATG CGC
 L   A   T   V   L   C   N   V   L   V   C   G   A   L   L   R   M   H   R   Q   F   M   R

CGC ACC TCG CTG GGC ACC GAG CAG CAC CAC GCG GCC GCG GCC GCC TCG GTT GCC TCC CGG GGC CAC CCC
 R   T   S   L   G   T   E   Q   H   H   A   A   A   A   A   S   V   A   S   R   G   H   P

GCT GCC TCC CCA GCC TTG CCG CGC CTC AGC GAC TTT CGG CGC CGC CGG AGC TTC CGC CGC ATC GCG GGC
 A   A   S   P   A   L   P   R   L   S   D   F   R   R   R   R   S   F   R   R   I   A   G

GCC GAG ATC CAG ATG GTC ATC TTA CTC ATT GCC ACC TCC CTG GTG GTG CTC ATC TGC TCC ATC CCG CTC
 A   E   I   Q   M   V   I   L   L   I   A   T   S   L   V   V   L   I   C   S   I   P   L

GTG GTG CGA GTA TTC GTC AAC CAG TTA TAT CAG CCA AGT TTG GAG CGA GAA GTC AGT AAA AAT CCA GAT
 V   V   R   V   F   V   N   Q   L   Y   Q   P   S   L   E   R   E   V   S   K   N   P   D

TTG CAG GCC ATC CGA ATT GCT TCT GTG AAC CCC ATC CTA GAC CCC TGG ATA TAT ATC CTC CTG AGA AAG
 L   Q   A   I   R   I   A   S   V   N   P   I   L   D   P   W   I   Y   I   L   L   R   K

ACA GTG CTC AGT AAA GCA ATA GAG AAG ATC AAA TGC CTC TTC TGC CGC ATT GGC GGG TCC CGC AGG GAG
 T   V   L   S   K   A   I   E   K   I   K   C   L   F   C   R   I   G   G   S   R   R   E

CGC TCC GGA CAG CAC TGC TCA GAC AGT CAA AGG ACA TCT TCT GCC ATG TCA GGC CAC TCT CGC TCC TTC
 R   S   G   Q   H   C   S   D   S   Q   R   T   S   S   A   M   S   G   H   S   R   S   F

ATC TCC CGG GAG CTG AAG GAG ATC AGC AGT ACA TCT CAG ACC CTC CTG CCA GAC CTC TCA CTG CCA GAC
 I   S   R   E   L   K   E   I   S   S   T   S   Q   T   L   L   P   D   L   S   L   P   D

CTC AGT GAA AAT GGC CTT GGA GGC AGG AAT TTG CTT CCA GGT GTG CCT GGC ATG GGC CTG GCC CAG GAA
 L   S   E   N   G   L   G   G   R   N   L   L   P   G   V   P   G   M   G   L   A   Q   E

GAC ACC ACC TCA CTG AGG ACT TTG CGA ATA TCA GAG ACC TCA GAC TCT TCA CAG GGT CAG GAC TCA GAG
 D   T   T   S   L   R   T   L   R   I   S   E   T   S   D   S   S   Q   G   Q   D   S   E

AGT GTC TTA CTG GTG GAT GAG GCT GGT GGG AGC GGC AGG GCT GGG CCT GCC CCT AAG GGG AGC TCC CTG
 S   V   L   L   V   D   E   A   G   G   S   G   R   A   G   P   A   P   K   G   S   S   L

CAA GTC ACA TTT CCC AGT GAA ACA CTG AAC TTA TCA GAA AAA TGT ATA TGA
Q   V   T   F   P   S   E   T   L   N   L   S   E   K   C   I
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS142M	ChemiScreen™ BB3 Bombesin receptor membrane prep

REFERENCES

1. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.
2. Nguyen M *et al.* (1997) The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature* 390: 78-81.
3. Yoshida K *et al.* (2002) Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *Proc. Natl. Acad. Sci. USA* 99: 4580-5.

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