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PRODUCT DATASHEET

Ready-to-Assay™ EP_2 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS185RTA

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 bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The protanoid PGE_2 causes pain, vasodilation, immunosuppression of T cells, bone resorption 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_2 couples primarily to G_s to increase intracellular cAMP levels. Mice deficient in EP_2 receptor showed impaired ovulation and fertilization, salt-sensitive hypertension (Kennedy *et al*., 1999). It has been shown that EP_2 receptors are also involved in cancer associated immunodeficiency. Thus, genetic knockout of the EP_2 receptor reduced tumor growth and prolonged survival in mice that had undergone isograft injection of MC26 or Lewis lung carcinoma cells (Yang *et al.*, 2003). Cloned human EP_2-expressing cell line is made in the Chem-9 host, which supports high levels of recombinant EP_2 expression on the cell surface and contains high levels of the promiscuous G protein 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_2

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.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

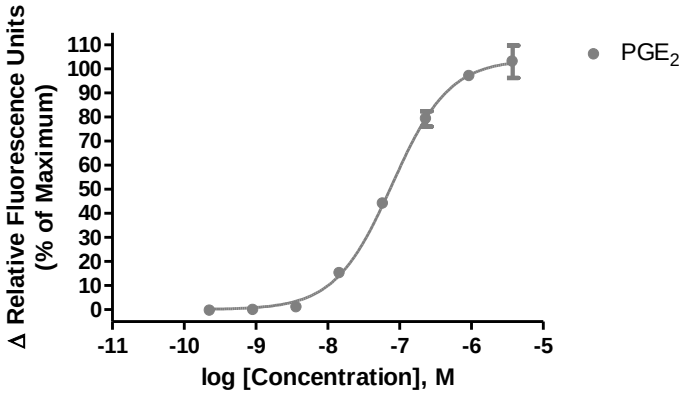

Figure 1. Representative data for activation of EP_2 receptor. Calcium flux in EP_2 –expressing Chem-9 cell line induced by Prostaglandin E_2 (EP_2). EP_2 –expressing Chem-9 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,800 RLU (Relative Light Units).

Table 1. EC_{50} values of EP_2 -expressing Chem-9 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
PDE_2	Calcium Flux	79	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-9, an adherent rat hematopoietic cell line expressing endogenous G·15 protein and a proprietary promiscuous G protein.

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EXONGENOUS GENE EXPRESSION

PTGER2 cDNA (Accession Number: NM_000956; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GGC AAT GCC TCC AAT GAC TCC CAG TCT GAG
                                                 M   G   N   A   S   N   D   S   Q   S   E

GAC TGC GAG ACG CGA CAG TGG CTT CCC CCA GGC GAA AGC CCA GCC ATC AGC TCC GTC ATG TTC TCG GCC
 D   C   E   T   R   Q   W   L   P   P   G   E   S   P   A   I   S   S   V   M   F   S   A

GGG GTG CTG GGG AAC CTC ATA GCA CTG GCG CTG CTG GCG CGC CGC TGG CGG GGG GAC GTG GGG TGC AGC
 G   V   L   G   N   L   I   A   L   A   L   L   A   R   R   W   R   G   D   V   G   C   S

GCC GGC CGC AGG AGC TCC CTC TCC TTG TTC CAC GTG CTG GTG ACC GAG CTG GTG TTC ACC GAC CTG CTC
 A   G   R   R   S   S   L   S   L   F   H   V   L   V   T   E   L   V   F   T   D   L   L

GGG ACC TGC CTC ATC AGC CCA GTG GTA CTG GCT TCG TAC GCG CGG AAC CAG ACC CTG GTG GCA CTG GCG
 G   T   C   L   I   S   P   V   V   L   A   S   Y   A   R   N   Q   T   L   V   A   L   A

CCC GAG AGC CGC GCG TGC ACC TAC TTC GCT TTC GCC ATG ACC TTC TTC AGC CTG GCC ACG ATG CTC ATG
 P   E   S   R   A   C   T   Y   F   A   F   A   M   T   F   F   S   L   A   T   M   L   M

CTC TTC GCC ATG GCC CTG GAG CGC TAC CTC TCG ATC GGG CAC CCC TAC TTC TAC CAG CGC CGC GTC TCG
 L   F   A   M   A   L   E   R   Y   L   S   I   G   H   P   Y   F   Y   Q   R   R   V   S

CGC TCC GGG GGC CTG GCC GTG CTG CCT GTC ATC TAT GCA GTC TCC CTG CTC TTC TGC TCG CTG CCG CTG
 R   S   G   G   L   A   V   L   P   V   I   Y   A   V   S   L   L   F   C   S   L   P   L

CTG GAC TAT GGG CAG TAC GTC CAG TAC TGC CCC GGG ACC TGG TGC TTC ATC CGG CAC GGG CGG ACC GCT
 L   D   Y   G   Q   Y   V   Q   Y   C   P   G   T   W   C   F   I   R   H   G   R   T   A

TAC CTG CAG CTG TAC GCC ACC CTG CTG CTG CTT CTC ATT GTC TCG GTG CTC GCC TGC AAC TTC AGT GTC
 Y   L   Q   L   Y   A   T   L   L   L   L   L   I   V   S   V   L   A   C   N   F   S   V

ATT CTC AAC CTC ATC CGC ATG CAC CGC CGA AGC CGG AGA AGC CGC TGC GGA CCT TCC CTG GGC AGT GGC
 I   L   N   L   I   R   M   H   R   R   S   R   R   S   R   C   G   P   S   L   G   S   G

CGG GGC GGC CCC GGG GCC CGC AGG AGA GGG GAA AGG GTG TCC ATG GCG GAG GAG ACG GAC CAC CTC ATT
 R   G   G   P   G   A   R   R   R   G   E   R   V   S   M   A   E   E   T   D   H   L   I

CTC CTG GCT ATC ATG ACC ATC ACC TTC GCC GTC TGC TCC TTG CCT TTC ACG ATT TTT GCA TAT ATG AAT
 L   L   A   I   M   T   I   T   F   A   V   C   S   L   P   F   T   I   F   A   Y   M   N

GAA ACC TCT TCC CGA AAG GAA AAA TGG GAC CTC CAA GCT CTT AGG TTT TTA TCA ATT AAT TCA ATA ATT
 E   T   S   S   R   K   E   K   W   D   L   Q   A   L   R   F   L   S   I   N   S   I   I

GAC CCT TGG GTC TTT GCC ATC CTT AGG CCT CCT GTT CTG AGA CTA ATG CGT TCA GTC CTC TGT TGT CGG
 D   P   W   V   F   A   I   L   R   P   P   V   L   R   L   M   R   S   V   L   C   C   R

ATT TCA TTA AGA ACA CAA GAT GCA ACA CAA ACT TCC TGT TCT ACA CAG TCA GAT GCC AGT AAA CAG GAC
 I   S   L   R   T   Q   D   A   T   Q   T   S   C   S   T   Q   S   D   A   S   K   Q   D

CTT TGA
 L  Stp
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS185M	ChemiScreen™ EP_2 Prostanoid receptor membrane prep

Note: Chem-9 cells are derived from Chem-1 cells.

REFERENCES

1. Kennedy CR *et al*.(1999) Salt-sensitivity hypertension and reduced fertility in mice lacking the prostaglandin EP_2 receptor. *Nat. Med.* 5:217-220.
2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.
3. Yang N *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP_2 receptor. *J. Clin. Invest.* 111: 727–735.

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