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PRODUCT DATASHEET

Ready-to-Assay™ EP_3 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS092RTA

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 subsequently 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 PGE2 causes pain, vasodilatation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP1, EP2, EP3 and EP4, each bind to PGE2, but the different G protein coupling status of each receptor leads to distinct biological effects. Further diversity is generated by alternative splicing; the human gene for EP3 generates 9 alternatively spliced mRNAs encoding 8 isoforms of EP3 (Kotani et al., 1997). These isoforms of EP3 vary in sequence at their C-termini, and differ in their ability to couple to Gs, Gq or Gi (Kotani et al., 1995). EP3 is required for fever induced by pyrogens, a response long attributed to prostaglandins by the antipyretic action of aspirin and other COX inhibitors (Ushikubi et al., 1998). In animal models of allergy, PGE2-mediated activation of EP3 inhibits inflammation to counteract the allergy-promoting activity of PGD2 (Kunikata et al., 2005). Cloned human EP_3-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant EP_3 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 EP_3.

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

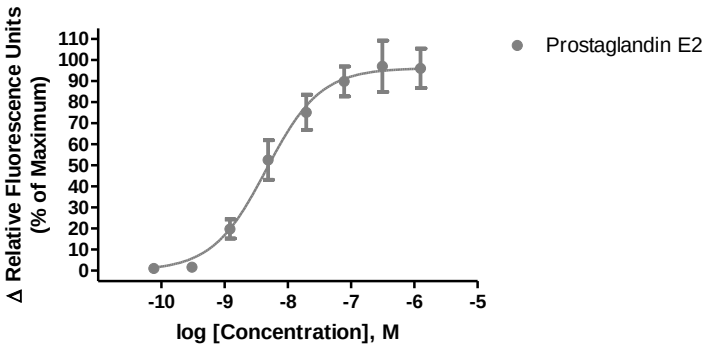

Figure 1. Representative data for activation of EP_3 receptor. Calcium flux in EP_3–expressing Chem-1 cell line induced by Prostaglandin E2. EP_3–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 26,000 RLU (Relative Light Units).

Table 1. Summary of EC_{50} values of EP_3-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin E2	Calcium Flux	4.6	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
Prostaglandin E2 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

PTGER3 cDNA (Accession Number: NM_198716; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG AAG GAG ACC CGG GGC TAC GGA GGG GAT GCC CCC
                                                 M   K   E   T   R   G   Y   G   G   D   A   P

TTC TGC ACC CGC CTC AAC CAC TCC TAC ACA GGC ATG TGG GCG CCC GAG CGT TCC GCC GAG GCG CGG GGC
 F   C   T   R   L   N   H   S   Y   T   G   M   W   A   P   E   R   S   A   E   A   R   G

AAC CTC ACG CGC CCT CCA GGG TCT GGC GAG GAT TGC GGA TCG GTG TCC GTG GCC TTC CCG ATC ACC ATG
 N   L   T   R   P   P   G   S   G   E   D   C   G   S   V   S   V   A   F   P   I   T   M

CTG CTC ACT GGT TTC GTG GGC AAC GCA CTG GCC ATG CTG CTC GTG TCG CGC AGC TAC CGG CGC CGG GAG
 L   L   T   G   F   V   G   N   A   L   A   M   L   L   V   S   R   S   Y   R   R   R   E

AGC AAG CGC AAG AAG TCC TTC CTG CTG TGC ATC GGC TGG CTG GCG CTC ACC GAC CTG GTC GGG CAG CTT
 S   K   R   K   K   S   F   L   L   C   I   G   W   L   A   L   T   D   L   V   G   Q   L

CTC ACC ACC CCG GTC GTC ATC GTC GTG TAC CTG TCC AAG CAG CGT TGG GAG CAC ATC GAC CCG TCG GGG
 L   T   T   P   V   V   I   V   V   Y   L   S   K   Q   R   W   E   H   I   D   P   S   G

CGG CTC TGC ACC TTT TTC GGG CTG ACC ATG ACT GTT TTC GGG CTC TCC TCG TTG TTC ATC GCC AGC GCC
 R   L   C   T   F   F   G   L   T   M   T   V   F   G   L   S   S   L   F   I   A   S   A

ATG GCC GTC GAG CGG GCG CTG GCC ATC AGG GCG CCG CAC TGG TAT GCG AGC CAC ATG AAG ACG CGT GCC
 M   A   V   E   R   A   L   A   I   R   A   P   H   W   Y   A   S   H   M   K   T   R   A

ACC CGC GCT GTG CTG CTC GGC GTG TGG CTG GCC GTG CTC GCC TTC GCC CTG CTG CCG GTG CTG GGC GTG
 T   R   A   V   L   L   G   V   W   L   A   V   L   A   F   A   L   L   P   V   L   G   V

GGC CAG TAC ACC GTC CAG TGG CCC GGG ACG TGG TGC TTC ATC AGC ACC GGG CGA GGG GGC AAC GGG ACT
 G   Q   Y   T   V   Q   W   P   G   T   W   C   F   I   S   T   G   R   G   G   N   G   T

AGC TCT TCG CAT AAC TGG GGC AAC CTT TTC TTC GCC TCT GCC TTT GCC TTC CTG GGG CTC TTG GCG CTG
 S   S   S   H   N   W   G   N   L   F   F   A   S   A   F   A   F   L   G   L   L   A   L

ACA GTC ACC TTT TCC TGC AAC CTG GCC ACC ATT AAG GCC CTG GTG TCC CGC TGC CGG GCC AAG GCC ACG
 T   V   T   F   S   C   N   L   A   T   I   K   A   L   V   S   R   C   R   A   K   A   T

GCA TCT CAG TCC AGT GCC CAG TGG GGC CGC ATC ACG ACC GAG ACG GCC ATT CAG CTT ATG GGG ATC ATG
 A   S   Q   S   S   A   Q   W   G   R   I   T   T   E   T   A   I   Q   L   M   G   I   M

TGC GTG CTG TCG GTC TGC TGG TCT CCG CTC CTG ATA ATG ATG TTG AAA ATG ATC TTC AAT CAG ACA TCA
 C   V   L   S   V   C   W   S   P   L   L   I   M   M   L   K   M   I   F   N   Q   T   S

GTT GAG CAC TGC AAG ACA CAC ACG GAG AAG CAG AAA GAA TGC AAC TTC TTC TTA ATA GCT GTT CGC CTG
 V   E   H   C   K   T   H   T   E   K   Q   K   E   C   N   F   F   L   I   A   V   R   L

GCT TCA CTG AAC CAG ATC TTG GAT CCT TGG GTT TAC CTG CTG TTA AGA AAG ATC CTT CTT CGA AAG TTT
 A   S   L   N   Q   I   L   D   P   W   V   Y   L   L   L   R   K   I   L   L   R   K   F

TGC CAG ATG AGA AAA AGA AGA CTC AGA GAG CAA GAG GAA TTT TGG GGA AAT TAA TGA
 C   Q   M   R   K   R   R   L   R   E   Q   E   E   F   W   G   N     Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS092C	ChemiScreen™ EP_3 Prostanoid receptor stable cell line
HTS092M	ChemiScreen™ EP_3 Prostanoid receptor membrane prep
HTS092L	ChemiBrite™ EP_3 Prostanoid receptor stable cell line

REFERENCES

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2. Kotani M et al. (1997) Structural Organization of the Human Prostaglandin EP3 Receptor Subtype Gene (PTGER3). *Genomics* 40: 425-434.
3. Kunikata T et al. (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat. Immunol*. 6: 524-531.
4. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest*. 108: 25-30.
5. Ushikubi F et al. (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395: 281-284.

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