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PRODUCT DATASHEET

Ready-to-Assay™ EP_1 Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS009RTA

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 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_1 couples primarily to G_q to mobilize intracellular calcium. EP_1 appears to mediate the effects of PGE_2 in promoting formation of precancerous lesions in animal models of colon cancer (Watanabe *et al*., 1999). In addition, EP_1 has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice (Matsuoka *et al*., 2005). Cloned human EP_1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant EP_1 expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at EP_1.

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

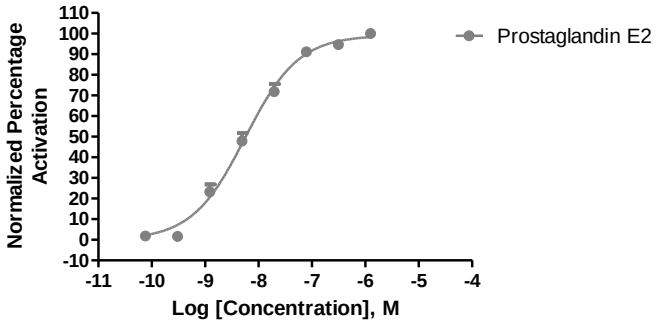

Figure 1. Representative data for activation of EP_1 receptor. Calcium flux in EP_1–expressing Chem-1 cell line induced by Prostaglandin E2. EP_1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 14,000 RLU (Relative Light Units).

Table 1. Summary of EC_{50} values of EP_1-expressing Chem-1 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin E2	Calcium Flux	7.5	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	Sigma: 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

PTGER1 cDNA (Accession Number: NM_000955; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG AGC CCT TGC GGG CCC CTC AAC CTG AGC CTG GCG
                                                 M   S   P   C   G   P   L   N   L   S   L   A

GGC GAG GCG ACC ACA TGC GCG GCG CCC TGG GTC CCC AAC ACG TCG GCC GTG CCG CCG TCG GGC GCT TCG
 G   E   A   T   T   C   A   A   P   W   V   P   N   T   S   A   V   P   P   S   G   A   S

CCC GCG CTG CCC ATC TTC TCC ATG ACG CTG GGC GCC GTG TCC AAC CTG CTG GCG CTG GCG CTG CTG GCG
 P   A   L   P   I   F   S   M   T   L   G   A   V   S   N   L   L   A   L   A   L   L   A

CAG GCC GCG GGC CGC CTG CGA CGC CGC CGC TCG GCC GCC ACC TTC CTG CTG TTC GTG GCC AGC CTG CTG
 Q   A   A   G   R   L   R   R   R   R   S   A   A   T   F   L   L   F   V   A   S   L   L

GCC ACC GAC CTG GCG GGC CAC GTG ATC CCG GGC GCG CTG GTG CTG CGT CTG TAC ACT GCG GGG CGC GCT
 A   T   D   L   A   G   H   V   I   P   G   A   L   V   L   R   L   Y   T   A   G   R   A

CCG GCC GGC GGG GCC TGC CAC TTC CTG GGC GGC TGC ATG GTC TTC TTC GGC CTG TGC CCG CTG CTG CTG
 P   A   G   G   A   C   H   F   L   G   G   C   M   V   F   F   G   L   C   P   L   L   L

GGC TGT GGC ATG GCC GTG GAG CGC TGC GTG GGC GTC ACG CGG CCG CTG CTC CAC GCC GCG CGG GTC TCG
 G   C   G   M   A   V   E   R   C   V   G   V   T   R   P   L   L   H   A   A   R   V   S

GTC GCC CGC GCG CGC CTG GCG CTG GCC GCG GTG GCC GCG GTG GCC TTG GCC GTG GCG CTG CTG CCG CTG
 V   A   R   A   R   L   A   L   A   A   V   A   A   V   A   L   A   V   A   L   L   P   L

GCG CGC GTG GGC CGC TAT GAG CTG CAG TAC CCG GGC ACG TGG TGC TTC ATC GGC CTG GGT CCC CCG GGC
 A   R   V   G   R   Y   E   L   Q   Y   P   G   T   W   C   F   I   G   L   G   P   P   G

GGC TGG CGC CAG GCA CTG CTT GCT GGC CTC TTC GCC AGC CTC GGC CTG GTC GCG CTC CTC GCC GCG CTG
 G   W   R   Q   A   L   L   A   G   L   F   A   S   L   G   L   V   A   L   L   A   A   L

GTG TGC AAC ACG CTC AGC GGC CTG GCC CTG CTA CGC GCC CGC TGG CGA CGC CGC TCC CGA CGG CCT CCC
 V   C   N   T   L   S   G   L   A   L   L   R   A   R   W   R   R   R   S   R   R   P   P

CCG GCC TCA GGC CCC GAC AGC CGG CGT CGC TGG GGG GCG CAC GGA CCC CGC TCG GCC TCC GCC TCG TCC
 P   A   S   G   P   D   S   R   R   R   W   G   A   H   G   P   R   S   A   S   A   S   S

GCC TCG TCC ATC GCT TCG GCC TCC ACC TTC TTT GGC GGC TCT CGG AGC AGC GGC TCG GCA CGC AGA GCT
 A   S   S   I   A   S   A   S   T   F   F   G   G   S   R   S   S   G   S   A   R   R   A

CGC GCC CAC GAC GTG GAG ATG GTG GGC CAG CTT GTC GGT ATC ATG GTG GTG TCG TGC ATC TGC TGG AGC
 R   A   H   D   V   E   M   V   G   Q   L   V   G   I   M   V   V   S   C   I   C   W   S

                                                                                    G→A
CCA ATG CTG GTG TTG GTG GCG CTG GCC GTC GGC GGC TGG AGC TCT ACC TCC CTG CAG CGG CCA CTA TTC
 P   M   L   V   L   V   A   L   A   V   G   G   W   S   S   T   S   L   Q   R   P L→L F

CTG GCC GTG CGC CTT GCC TCC TGG AAC CAG ATC CTG GAC CCT TGG GTG TAC ATC CTA CTG CGC CAG GCC
 L   A   V   R   L   A   S   W   N   Q   I   L   D   P   W   V   Y   I   L   L   R   Q   A

GTG CTG CGC CAA CTG CTT CGC CTC TTG CCC CCG AGG GCC GGA GCC AAG GGC GGC CCC GCG GGG CTG GGC
 V   L   R   Q   L   L   R   L   L   P   P   R   A   G   A   K   G   G   P   A   G   L   G

CTA ACA CCG AGC GCC TGG GAG GCC AGC TCG CTG CGC AGC TCC CGG CAC AGC GGC CTC AGC CAC TTC TAA
 L   T   P   S   A   W   E   A   S   S   L   R   S   S   R   H   S   G   L   S   H   F

TGA
Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS099C	ChemiScreen™ EP_1 Prostanoid receptor stable cell line
HTS099M	ChemiScreen™ EP_1 Prostanoid receptor membrane prep
HTS099L	ChemiBrite™ EP_1 Prostanoid receptor stable cell line
HTS099LT	ChemiBrite™ EP_1 Prostanoid receptor frozen cells

REFERENCES

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2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest*. 108: 25-30.
3. Watanabe K et al.(1999) Role of prostaglandin E receptor subtype EP1 in colon carcinogenesis. *Cancer Res*. 59: 5093-5096.

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