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PRODUCT DATASHEET

ChemiScreen™ EP_4 Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS142C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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). The cloned human EP_4-expressing cell line is 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 **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

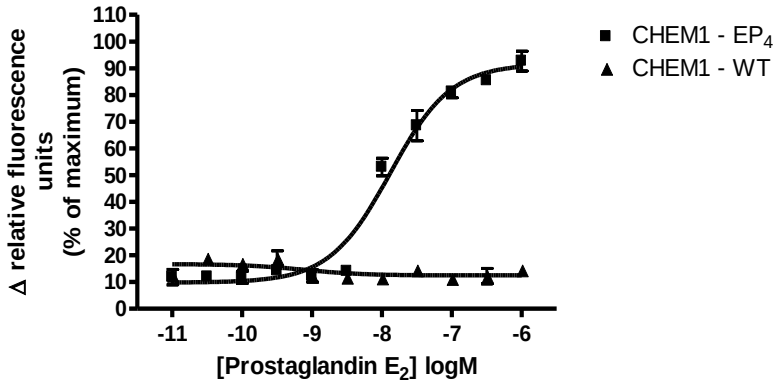

Figure 1. Representative data for activation of the EP_4 receptor stably expressed in Chem-1 cells induced by PGE_2 using a fluorescent calcium flux assay. EP_4–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
PGE_2	Calcium Flux - Fluorescence	13	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
PGE_2 ligand	Cayman: 14010
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human EP_4 cDNA (Accession Number: NM_000958; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

```
                                                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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS142M	ChemiScreen™ EP_4 Prostanoid 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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