

## PRODUCT DATASHEET

### ChemiScreen™ EP<sub>3</sub> Prostanoid Receptor Stable Cell Line

#### CATALOG NUMBER: HTS092C

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

**STORAGE:** Vials are to be stored in liquid N<sub>2</sub>.

#### 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 Gα<sub>15</sub>, 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 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<sub>2</sub> causes pain, vasodilatation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>, each bind to PGE<sub>2</sub>, 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 EP<sub>3</sub> generates 9 alternatively spliced mRNAs encoding 8 isoforms of EP<sub>3</sub> (Kotani *et al.*, 1997). These isoforms of EP<sub>3</sub> vary in sequence at their C-termini, and differ in their ability to couple to G<sub>s</sub>, G<sub>q</sub> or G<sub>i</sub> (Kotani *et al.*, 1995). EP<sub>3</sub> 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, PGE<sub>2</sub>-mediated activation of EP<sub>3</sub> inhibits inflammation to counteract the allergy-promoting activity of PGD<sub>2</sub> (Kunikata *et al.*, 2005). The cloned human EP<sub>3</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant EP<sub>3</sub> expression on the cell surface and contains high levels of the promiscuous G protein Gα<sub>15</sub> to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between EP<sub>3</sub> and its ligands.

#### USE RESTRICTIONS

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#### 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.

## APPLICATIONS

Calcium Flux Fluorescence Assay

## APPLICATION DATA

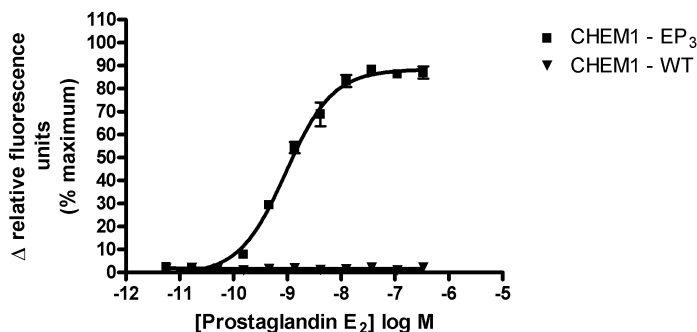


Figure 1. Representative data for activation of the EP<sub>3</sub> receptor stably expressed in Chem-1 cells induced by PGE<sub>2</sub> using a fluorescent calcium flux assay. EP<sub>3</sub>-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells 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<sup>TETRA</sup>® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 9,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC<sub>50</sub> value of EP<sub>3</sub>-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC <sub>50</sub> (nM)	REFERENCE
PGE <sub>2</sub>	Calcium Flux - Fluorescence	1	Eurofins Internal Data

\* The cell line was tested and found to have equivalent EC<sub>50</sub> 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
<b>Basal Medium</b>	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
<b>Selection Medium</b>	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
<b>Dissociation</b>	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
<b>CryoMedium</b>	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 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C 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, 37°C, 5% CO<sub>2</sub>.
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 37° 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 <sup>2</sup> )	Volume (mL)	Total Cell Number (x10 <sup>6</sup> )	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.6	72

## ASSAY SETUP

### Fluorescence

Table 4. Settings for FLIPR<sup>TETRA</sup>® 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 <sub>2</sub> 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  $5 \times 10^5$  cells/ml (i.e, if collected  $5 \times 10^6$  TC,  $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$  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 37°C 5% CO<sub>2</sub> 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<sup>TETRA</sup>® 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<sub>3</sub> cDNA (Accession Number: NM\_198716; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

## 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 A V L A F A L L P V L G V
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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS092M	ChemiScreen™ EP <sub>3</sub> Prostanoid Receptor Membrane Prep

## REFERENCES

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2. Kotani M *et al.* (1997) Structural Organization of the Human Prostaglandin EP<sub>3</sub> Receptor Subtype Gene (PTGER3). *Genomics* 40: 425-434.
3. Kunikata T *et al.* (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP<sub>3</sub>. *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 EP<sub>3</sub>. *Nature* 395: 281-284.

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