

PRODUCT DATASHEET

ChemiScreen™ EP₁ Prostanoid Receptor Stable Cell Line

CATALOG NUMBER: HTS099C

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

STORAGE: Vials are to be stored in liquid N₂.

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 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, vasodilation, 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; EP₁ couples primarily to G_q to mobilize intracellular calcium. EP₁ appears to mediate the effects of PGE₂ in promoting formation of precancerous lesions in animal models of colon cancer (Watanabe *et al.*, 1999). In addition, EP₁ has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice (Matsuoka *et al.*, 2005). The cloned human EP₁-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant EP₁ expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between EP₁ and its ligands.

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.

APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

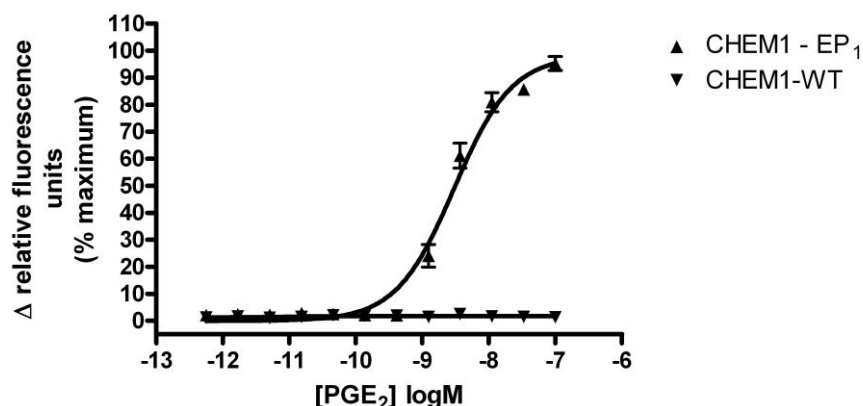


Figure 1. Representative data for activation of EP₁ receptor stably expressed in **Chem-1** cells induced by PGE₂ using a fluorescent calcium flux assay. EP₁-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC₅₀ value of EP₁-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
PGE ₂	Calcium Flux - Fluorescence	3	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC₅₀ 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 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% CO₂.
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 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 TM , 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 5x10⁵ cells/ml (i.e, if collected 5e6 TC, $\frac{5e6}{5e5/ml} = 10$ 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 37°C 5% CO₂ 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^{TE1RA}® 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 EP₁ cDNA (Accession Number: NM_000955; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

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 GGC 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

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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 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

 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

RELATED PRODUCTS

Product Number

Description

HTSCHEM-1

ChemiScreen™ Chem-1 Parental Cell Line (control cells)

HTS099M

 ChemiScreen™ EP₁ Prostanoid Receptor Membrane Prep

REFERENCES

1. Matsuoka Y et al. (2005) Prostaglandin E receptor EP₁ controls impulsive behavior under stress. *Proc. Natl. Acad. Sci. USA*. 102: 16066-16071.
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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