

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT EP<sub>3</sub> PROSTANOID RECEPTOR**

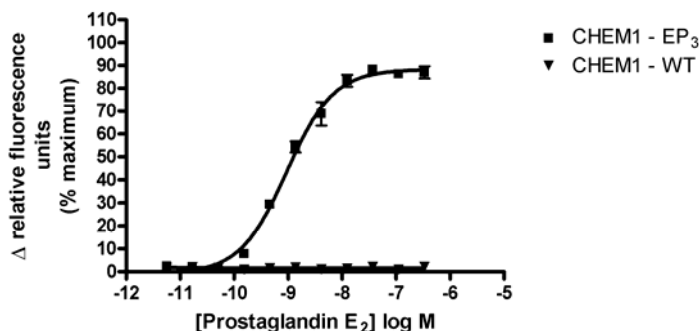
<b>CATALOG NUMBER:</b>	HTS092C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:**

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). Millipore's 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 G<sub>α</sub>15 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.

**APPLICATIONS:**

Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in EP<sub>3</sub>-expressing Chem-1 cell line induced by PGE<sub>2</sub>. EP<sub>3</sub>-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 NW and calcium flux in response to PGE<sub>2</sub> (10<sup>-6.5</sup> to 10<sup>-11.5</sup> M) was determined in triplicate on a Molecular Devices FLIPR<sup>TETRA</sup>™.

**SPECIFICATIONS:** EC<sub>50</sub> for calcium mobilization by PGE<sub>2</sub>: ~ 1 nM  
Signal/noise ratio at ligand E<sub>max</sub>: 399

**HOST CELLS:** Chem-1, an adherent cell line expressing the promiscuous G-protein, G<sub>α</sub>15.

TRANSFECTION: Full-length human PTGER3 cDNA encoding splice variant 6 of EP<sub>3</sub>  
(Accession Number: NM\_198716)

**PRESENTATION:**

Cells are frozen at  $2 \times 10^6$  cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

**STORAGE/HANDLING:**

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub>.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca<sup>++</sup> and Mg<sup>++</sup> (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO<sub>2</sub> until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at  $5 \times 10^6$  cells/mL in Chem-1 Freezing Media (cell densities of  $2-10 \times 10^6$  are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Chem-1 Plating Media for plating for calcium assay.

**MEDIA:**

## Chem-1 Growth Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)  
10% heat-inactivated FBS  
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)  
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)  
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)  
250µg/mL Genetecin/G-418

## Chem-1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine  
10% heat-inactivated FBS  
1x NEAA  
10mM HEPES  
1x Pen-Strep

Chem-1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine  
20% heat-inactivated FBS  
1x NEAA  
10mM HEPES  
1x Pen-Strep  
10% DMSO (cell culture grade)

**REFERENCES:**

Kotani M *et al.* (1995) Molecular cloning and expression of multiple isoforms of human prostaglandin E receptor EP3 subtype generated by alternative messenger RNA splicing: multiple second messenger systems and tissue-specific distributions. *Mol Pharmacol.* 48: 869-879.

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Kunikata T *et al.* (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP<sub>3</sub>. *Nat. Immunol.* 6: 524-531.

Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

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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