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ChemiScreen™ FLASH AEQUORIN CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT EP_3 PROSTANOID RECEPTOR

CATALOG NUMBER:	HTS092AF	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	R0711E0005	**CONCENTRATION:**	2 x 10^6 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_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. Further diversity is generated by alternative splicing; the human gene for EP_3 generates 9 alternatively spliced mRNAs encoding 8 isoforms of EP_3 (Kotani *et al.*, 1997). These isoforms of EP_3 vary in sequence at their C-termini, and differ in their ability to couple to G_s, G_q or G_i (Kotani *et al.*, 1995). EP_3 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_2-mediated activation of EP_3 inhibits inflammation to counteract the allergy-promoting activity of PGD_2 (Kunikata *et al.*, 2005). Millipore's cloned human EP_3 -expressing cell line is made in the Chem-1 host which stably expresses a mitochondrially targeted flash mutant form of aequorin. The purified version of this flash variant of aequorin has shown a higher luminescent signal intensity than purfied wildtype aequorin. Thus, the cell line is an ideal tool for screening for antagonists of interactions between EP_3 and its ligands.

APPLICATIONS:

Luminescent and fluorescent calcium flux assays, ligand binding assays

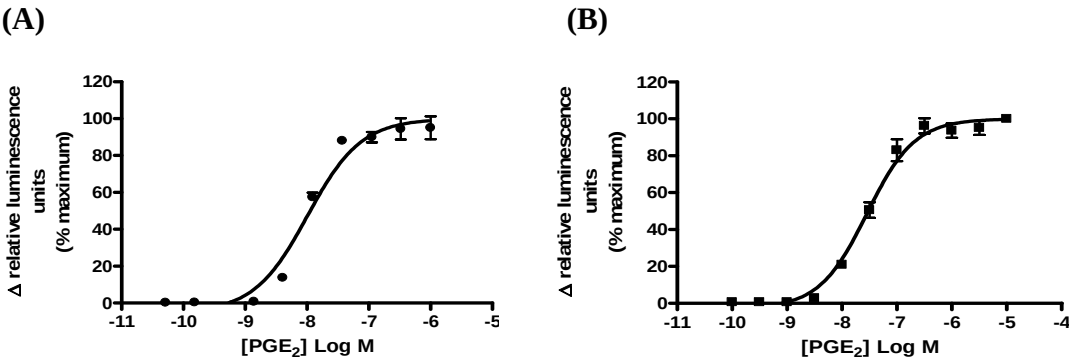

Figure 1. Ligand-induced calcium flux in Flash Aequorin Chem-1 cell line stably transfected with EP_3. Flash Aequorin Chem-1 stably co-expressing EP_3 were loaded with 5 μM coelenterazine for 3 h at room temperature. Luminescence in response to PGE_2 was determined (A) in quadruplicate in a 384 well plate on a FLIPR^{TETRA} with aequorin option from Molecular Devices, now part of MDS Analytical Technologies. Data were collected for area under curve for 70 sec. (B) PGE_2-induced luminescence was determined in duplicate in a 96 well plate on a PerkinElmer Wallac Victor2. Data were collected for area under curve for 20 sec.

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SPECIFICATIONS: EC50 for calcium mobilization by PGE_2: ~ 10.2 nM ($FLIPR^{TETRA}$)
~ 28.3 nM (Wallac Victor2)

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

TRANSFECTION: Full-length human PTGER3 cDNA encoding splice variant 6 of EP_3 (Accession Number: NM_198716)

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% fetal bovine serum/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 37ºC 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 37ºC with 5% CO_2.
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^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (2-4 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Aequorin 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 x 10^6 cells/mL in Freezing Media (cell densities of 2-10 x 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.

MEDIA:

Chem-1 Aequorin 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)
10 mM 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
250μg/mL Hygromycin

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

Freezing Media:
90% heat-inactivated FBS
10% DMSO (cell culture grade)

RECOMMENDED ASSAY CONDITIONS:

1. Seed cells in 96-well white plate (top-read instruments) or opaque-walled (bottom-read instruments) overnight at 50,000 cells/well in Chem-1 Aequorin Plating Media.
2. Wash cells once (200 µl/well) with Wash Buffer (HBSS with Ca^{++} and Mg^{++} containing 10 mM HEPES) before loading with 5 µM of coelenterazine (Millipore ES016) in wash buffer at room temperature for 3 hours.

   Note: *Luminescence activity has been determined to be optimal at room temperature. Incubation at 37°C will result in substantially reduced signals.*

3. After loading, wash cells once with Wash Buffer (200 µl/well) prior to addition of ligands.

REFERENCE:

Kotani M *et al.* (1995) Molecular cloning and expression of multiple isoforms of human prostaglandin E receptor EP_3 subtype generated by alternative messenger RNA splicing: multiple second messenger systems and tissue-specific distributions. *Mol. Pharmacol.* 48: 869-879.

Kotani M *et al.* (1997) Structural Organization of the Human Prostaglandin EP_3 Receptor Subtype Gene (PTGER3). *Genomics* 40: 425-434

Kunikata T *et al*. (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP_3. *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_3. *Nature* 395: 281-284.

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