

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT EP₂ PROSTANOID RECEPTOR**

CATALOG NUMBER:	HTS185F	QUANTITY:	1 vial, 1 mL
LOT NUMBER:		CONCENTRATION:	1 x 10 ⁷ viable cells/mL

BACKGROUND: Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay™ cells is identical to that of the originating GPCR cell line.

Prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostanoid 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. EP₂ couples primarily to G_s to increase intracellular cAMP levels. Mice deficient in EP₂ receptor showed impaired ovulation and fertilization, salt-sensitive hypertension (Kennedy *et al.*, 1999). It has been shown that EP₂ receptors are also involved in cancer associated immunodeficiency. Thus, genetic knockout of the EP₂ receptor reduced tumor growth and prolonged survival in mice that had undergone isograft injection of MC26 or Lewis lung carcinoma cells (Yang *et al.*, 2003). Millipore's cloned EP₂-expressing cell line is made in the Chem-9 host, an adherent cell line that supports high levels of recombinant EP₂ expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated EP₂-Chem-9 cell line and the Ready-To-Assay™ EP₂ cells have equivalent EC₅₀s for PGE₂.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC ₅₀ for PGE ₂ (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	30.7	1532	0.63
Continuous Passage Cells	25.5	2120	0.74

HOST CELLS: Chem-9, an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human PTGER2 cDNA encoding EP₂ (Accession Number: NM_000956)

PLATING 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)

PRESENTATION:

Cells are frozen at 1×10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

- 1) 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.
- 2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
- 3) Resuspend cells to 0.5×10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 μ L per well to obtain a density of approximately 1×10^5 cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
- 5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

Kennedy CR *et al.* (1999) Salt-sensitivity hypertension and reduced fertility in mice lacking the prostaglandin EP₂ receptor. *Nat. Med.* 5:217-220.

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

Yang N *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP₂ receptor. *J. Clin. Invest.* 111: 727-735.

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READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT EP₂ PROSTANOID RECEPTOR
Product No. HTS185F**

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