

**READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT IP₁ PROSTANOID RECEPTOR**

CATALOG NUMBER:	HTS131F	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 G α 15 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.

Prostacyclin (PGI₂) is released by vascular endothelial cells and serves as a potent vasodilator, inhibitor of platelet aggregation, and moderator of vascular smooth muscle cell proliferation–migration–differentiation (Narumiya *et al.* 1999). The function of prostacyclin is mediated via a seven transmembrane GPCR IP₁, which is known to couple to G_s and G_q signaling pathways. Mice lacking the IP₁ receptor have shown increased susceptibility to thrombosis (Murata *et al.* 1997), enhanced injury-induced vascular proliferation and platelet activation (Cheng *et al.* 2002), as well as reperfusion injury (Xiao *et al.* 2001). The recent world-wide withdrawal of selective COX-2 inhibitors, rofecoxib (Vioxx™) and valdecoxib (Bextra™), is also due to their discriminating suppression of COX-2-derived prostacyclin and IP₁-mediated cardioprotective effects, leading to increased risk of cardiovascular events (Fitzgerald 2004). Millipore cloned human IP₁-expressing cell line is made in the Chem-1 host, an adherent cell line that supports high levels of recombinant IP₁ expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. The untreated IP₁-Chem-1 cell line and the Ready-To-Assay IP₁ cells have equivalent EC50s for Iloprost.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC50 for Iloprost (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	11.8	5167	0.54
Continuous Passage Cells	21.8	6154	0.47

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

Gα15.

TRANSFECTION: Full-length human human PTGIR cDNA encoding IP₁ (Accession Number: NM_000960)

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 μL 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:

Narumiya S *et al.* (1999) Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79: 1193–1226.

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Cheng Y *et al.* (2002) Role of prostacyclin in the cardiovascular response to thromboxane A₂. *Science* 296: 539–541.

Xiao CH *et al.* (2001) Roles of prostaglandin I₂ and thromboxane A₂ in cardiac ischemia-reperfusion injury: a study using mice lacking their respective receptors. *Circulation* 104: 2210–2215

Fitzgerald GA (2004) Coxibs and cardiovascular disease, *N. Engl. J. Med.* 351: 1709–1711

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HUMAN RECOMBINANT IP₁ PROSTANOID RECEPTOR
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