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READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT IP_1 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 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.

Prostacyclin (PGI_2) 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 protacyclin is mediated via a seven transmembrane GPCR IP1, which is known to couple to Gs and Gq signaling pathways. Mice lacking the IP_1 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 IP_1-mediated cardioprotective effects, leading to increased risk of cardiovascular events (Fitzgerald 2004). Millipore cloned human IP_1-expressing cell line is made in the Chem-1 host, an adherent cell line that supports high levels of recombinant IP_1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. The untreated IP_1-Chem-1 cell line and the Ready-To-Assay™ IP_1 cells have equivalent EC50s for Iloprost.

APPLICATIONS:

Calcium flux assay

SPECIFICATIONS:

	EC50 for Iloprost (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	12	5167	0.54
Continuous Passage Cells	22	6154	0.47

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

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Gα15.

TRANSFECTION: Full-length human human PTGIR cDNA encoding IP_1 (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 x 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 37ºC 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 x 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 x 10^5 cells/well.
4) Place the assay plate in a humidified 37°C incubator with 5% CO_2.
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.

Murata T *et al.* (1997) Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* 388: 678–682.

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 I_2 and thromboxane A_2 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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GMO

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READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT IP_1 PROSTANOID RECEPTOR
Product No. HTS131F

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