eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ IP1
Prostanoid Receptor Frozen Cells

CATALOG NUMBER: HTS131RTA
Lot: 01022016

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. The freezing conditions have been optimized to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

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 protacyclin is mediated via a seven transmembrane GPCR, IP1, which is known to couple to Gs and Gq 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). The cloned human IP1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant IP1 expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between IP1 and its ligands.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Eurofins Pharma Bioanalytics Services US Inc.
6 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 441 6058
www.eurofins.com

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

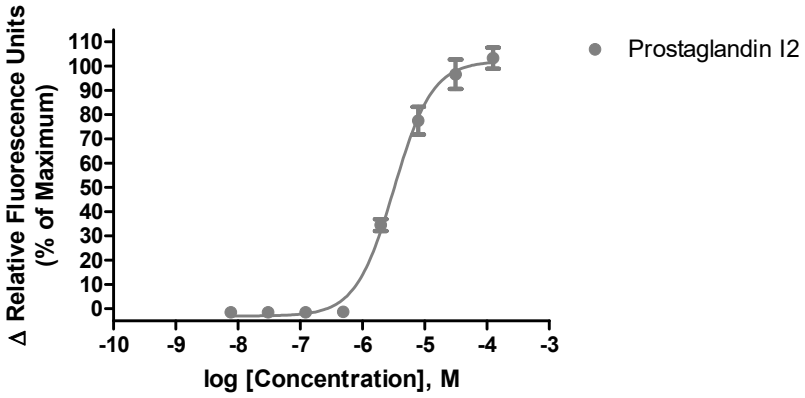

Figure 1. Representative data for activation of IP1 receptor. Calcium flux in IP1–expressing Chem-1 cell line induced by Prostaglandin I2. IP1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} equipped with ICCD camera. Maximal fluorescence signal obtained in this experiment was 23,000 RLU (Relative Light Units)..

Table 1. Comparison of EC_{50} values of IP1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Prostaglandin I2	Calcium Flux	3200	Eurofins Internal Data

ASSAY SETUP

Fluorescence

Table 3. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 4. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C

Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Prostaglandin I2	Various
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

HOST CELL

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

EXONGENOUS GENE EXPRESSION

Human IP1 cDNA (Accession Number: NM_000960; see CODING SEQUENCE below) expressed from a proprietary E5 promoter plasmid

CODING SEQUENCE

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atggcggattcgtgcaggaacctcacctacgtgcggggctcggtggggccggccaccagc
M  A  D  S  C  R  N  L  T  Y  V  R  G  S  V  G  P  A  T  S
accctgatgttcgtggccggtgtggtgggcaacgggctggccctgggcatcctgagcgca
T  L  M  F  V  A  G  V  V  G  N  G  L  A  L  G  I  L  S  A
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V  A  C  S  L  C  -
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)

REFERENCES

1. Narumiya S, Sugimoto Y and Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. Physiol. Rev. 79: 1193–1226.

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3. Cheng Y, Austin SC, Rocca B et al. (2002) Role of prostacyclin in the cardiovascular response to thromboxane A2. Science 296: 539–541.

4. Xiao CH, Hara A, Yuhki KI 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.

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

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