Human Recombinant Prostanoid IP1 Receptor Stable Cell Line

Technical Manual No. TM0506 **Version 10132010**

I. Introduction

Catalog Number: M00310

Cell Line Name: CHO-K1/IP1/Gα15

Gene Synonyms: IP; PRIPR; MGC102830; PTGIR

Expressed Gene: Genbank Accession Number NM_000960; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for IP1 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 100 μg/ml Hygromycin B, 400 μg/ml G418.

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. Background

IP1 receptor is a receptor for prostaglandin I2 or prostacyclin. When binding with a prostacyclin molecule, the receptor changes conformation and activates Gs. Prostaglandin I2, the major product of cyclooxygenase in macrovascular endothelium, mediates a potent vasodilation and inhibition of platelet aggregation by binding to this receptor.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. neurolyticum, M. hyopneumoniae and M. capricolum) and one species Ureaplasma (U. urealyticum), with sufficient sensitivity and specificity.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Iloprost in CHO-K1/IP1/Gα15 and CHO-K1/Gα15 cells

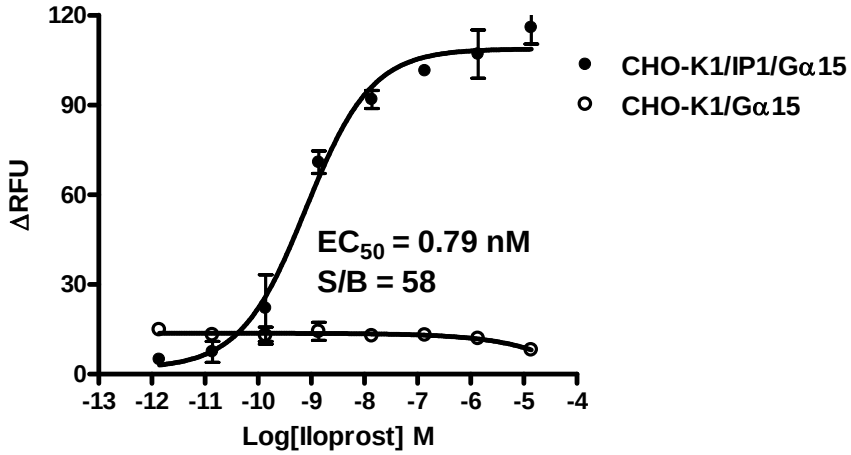

Figure 1. Iloprost-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/IP1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an IP1 receptor agonist, Iloprost. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Iloprost (Mean ± SD, n = 2). The EC_{50} of Iloprost on IP1 co-expressing with Gα15 in CHO-K1 cells was 0.79 nM. The S/B of Iloprost on IP1 co-expressing with Gα15 in CHO-K1 cells was 58.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))

   X is the logarithm of concentration. Y is the response

   Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.

2. Signal to background Ratio (S/B) = Top/Bottom

IV. Thawing and Subculturing

Thawing: Protocol

1. Remove the vial from liquid nitrogen tank and thaw the cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and G418 to concentrations of 100 µg/ml and 400 µg/ml respectively the following day.

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Subculturing: Protocol

1. Remove and discard culture medium.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution to a10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
   Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

1. Boie, Y., Rushmore, TH., Darmon-Goodwin, A., *et al.* (1994) Cloning and expression of a cDNA for the human prostanoid IP receptor. *J. Biol. Chem.* 269 (16): 12173–12178.
2. Katsuyama, M., Sugimoto, Y., Namba, T., *et al.* (1994) Cloning and expression of a cDNA for the human prostacyclin receptor. *FEBS Lett.* 344 (1): 74–78.
3. Coleman, RA., Smith, WL., Narumiya, S. (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* 46 (2): 205–229.

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