Human Recombinant Prostanoid Receptor DP1 Stable Cell Line

Technical Manual No. TM0611 **Version 11172010**

I. Introduction

Catalog Number: M00453

Cell Line Name: CHO-K1/DP1/Gα15

Gene Synonyms: PTGDR, AS1, ASRT1, DP, MGC49004

Expressed Gene: Genbank Accession Number NM_000953; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: 2 vial (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for DP1 receptor

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

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

Culture Medium: Ham's F12, 10% FBS, 100 μg/ml Hygromycin B, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

DP1 is a G_S-coupled GPCR expressed in the colon, eye, platelets, eosinophils, that bind to PGD2 and PGE2. DP1 can inhibit platelet aggregation, and also inhibit leukotriene B4 and superoxide anion (O2-) release from human neutrophils. It can relax smooth muscle and regulate eosinophil apoptosis. DP receptor knockout OVA-challenged mice exhibit reduced Th2 cytokine levels and lymphocyte accumulation in the lung. In addition, they failed to produce asthmatic responses.

§: 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.

860 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by PGD2 in CHO-K1/DP1/Gα15 and CHO-K1/Gα15 cells

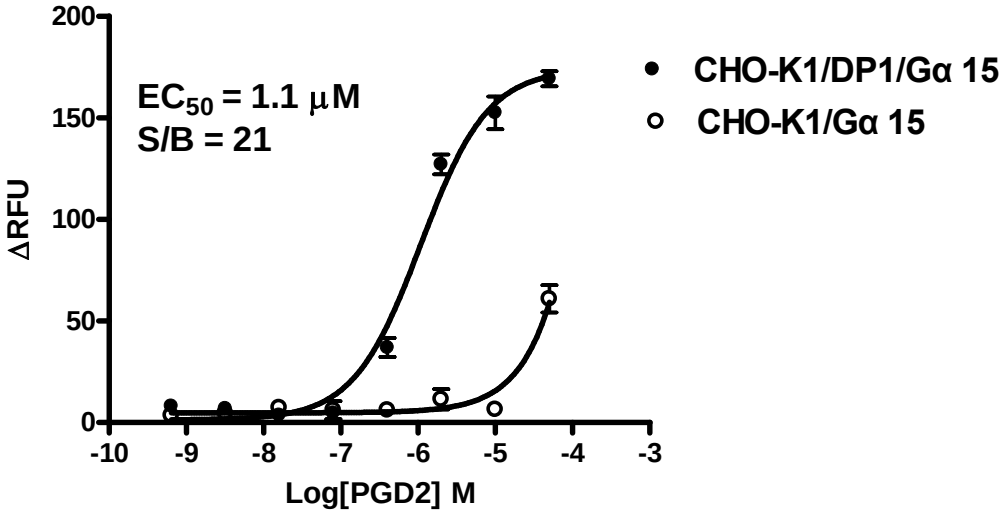

Figure 1. PGD2-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/DP1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a DP1 receptor agonist, PGD2. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of PGD2 (Mean ± SD, n = 2). The EC_{50} of PGD2 on DP1 co-expressing with Gα15 in CHO-K1 cells was 1.1 μM. The S/B of PGD2 on DP1 co-expressing with Gα15 in CHO-K1 cells was 21.

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 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 force 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 Zeocin to concentrations of 100 μg/ml and 200 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 10 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 force 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. Keery RJ, Lumley P. (1988) AH6809, a prostaglandin DP-receptor blocking drug on human platelets. *Br J Pharmacol.* 94(3):745-54.
2. Moreland RB *et. al.* (2002) Expression of functional prostaglandin D (DP) receptors in human corpus cavernosum smooth muscle. *Int J Impot Res.* 14(6):446-52.
3. Wheeldon A, Vardey CJ. (1993) Characterization of the inhibitory prostanoid receptors on human neutrophils. *Br J Pharmacol.* 108(4):1051-4.
4. Wright DH *et al*. (2000) The human prostanoid DP receptor stimulates mucin secretion in LS174T cells. *Br J Pharmacol.* 131(8):1537-45.

GenScript USA Inc.
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com

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860 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com