Human Recombinant Prostanoid Receptor DP2 Stable Cell Line

Technical Manual No. TM0563 **Version 10132010**

I. Introduction

Catalog Number: M00436

Cell Line Name: CHO-K1/DP2/Gα15

Gene Synonyms: GPR44, CRTH2

Expressed Gene: Genbank Accession Number NM_004778; 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 DP2 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

Prostaglandin D(2) (PGD(2)) is derived from arachidonic acid and binds with high affinity to the G-protein coupled receptors prostanoid DP(1) and DP(2). Interaction with DP(2) results in cell chemotaxis, eosinophil degranulation, eosinophil shape change, adhesion molecule upregulation and Th2 cytokine production. In allergic rhinitis and allergic asthma PGD(2) is released from mast cells in response to allergen challenge and may trigger symptoms such as sneezing, rhinorrhea, pruritus, mucus hypersecretion and pulmonary inflammation. GenScript's cloned human DP2-expressing cell line co-expressing Gα15 is made in the CHO-K1 host.

§: 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 PGD2 in CHO-K1/DP2/Gα15 and CHO-K1/Gα15 cells

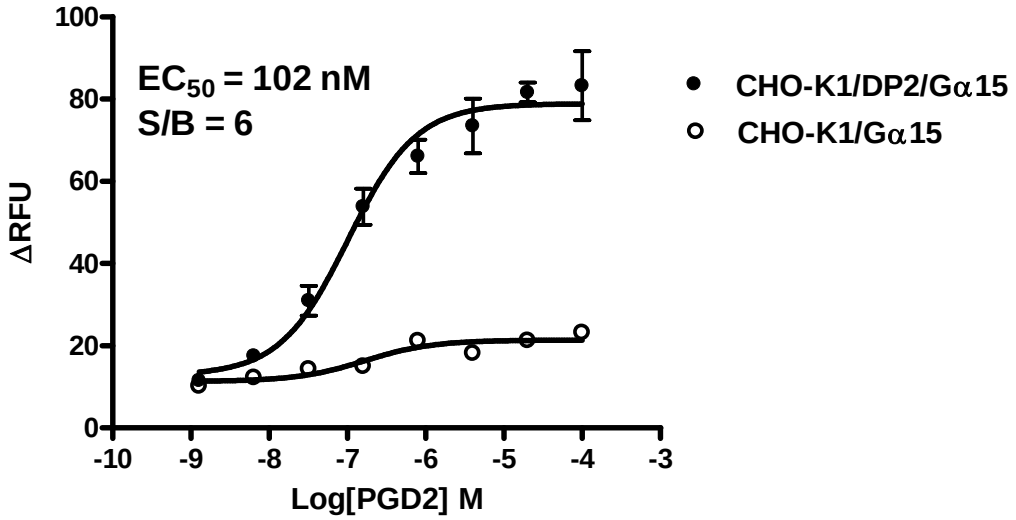

Figure 1. PGD2-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/DP2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a DP2 receptor agonist, PGD2. The intracellular calcium change was measured by FlexStation. The relative 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 DP2 co-expressing with Gα15 in CHO-K1 cells was 102 nM. The S/B of PGD2 on DP2 co-expressing with Gα15 in CHO-K1 cells was 6.

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 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 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. Stebbins KJ *et al.* (2010) Therapeutic efficacy of AM156, a novel prostanoid DP2 receptor antagonist, in murine models of allergic rhinitis and house dust mite-induced pulmonary inflammation. *Eur J Pharmacol.* 638(1-3):142-9
2. Mathiesen JM *et al.* (2006) On the mechanism of interaction of potent surmountable and insurmountable antagonists with the prostaglandin D2 receptor CRTH2. *Mol Pharmacol*. 69:1441–1453

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