Human Recombinant Prostanoid Receptor DP2 Stable Cell Line
Cat. No. M00436 **Version 07302020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for DP2 receptor
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F12 (Cat. #11765, Life Technologies), 10% FBS
Culture Medium: Ham's F12, 10% FBS, 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen), 3 μg/ml puromycin (Cat. #A11138-03, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by PGD2 in CHO-K1/DP2/Gα15 cells

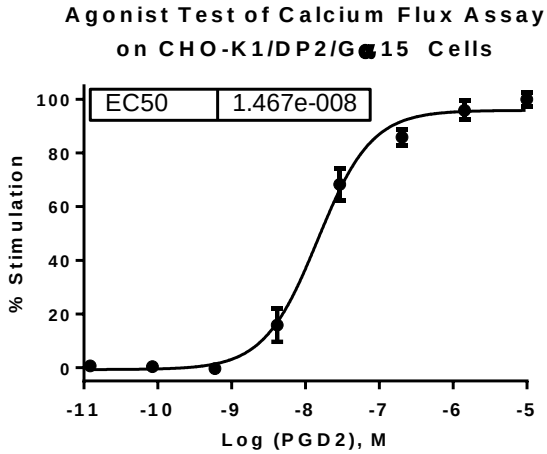

Figure 1. PGD2-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/DP2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist, PGD2. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of PGD2 (Mean ± SD, n = 2). The EC_{50} of PGD2 on this cell was 15 nM.

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 remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 %CO_2.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.

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 into 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 cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO_2.

Subcultivation Ratio: 1:3 to 1:8
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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