Human Recombinant PTGER2 Prostanoid Receptor Stable Cell Line
Cat. No. M00311 **Version 07292020**

I. INTRODUCTION

Catalog Number: M00311
Cell Line Name: CHO-K1/PTGER2/Gα15
Gene Synonyms: EP2
Expressed Gene: Genbank Accession Number NM_000956; 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 PTGER2 receptor (Calcium flux assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 µg/ml Geneticin (Cat. #10131-035, Life Technologies), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

Prostaglandin (PG) E2 exerts its actions by acting on a group of G-protein-coupled receptors (GPCRs). There are four GPCRs responding to PGE2 designated subtypes EP1, EP2, EP3, and EP4 and multiple splicing isoforms of the subtype EP3. The EP subtypes exhibit differences in signal transduction, tissue localization, and expression regulation. Studies have identified that EP2 mediates many processes, such as facilitating ovulation and fertilization, suppressing dendritic cell differentiation, and promoting amyloid-β formation in Alzheimer's disease.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by PGE2 in CHO-K1/PTGER2/Gα15 cells

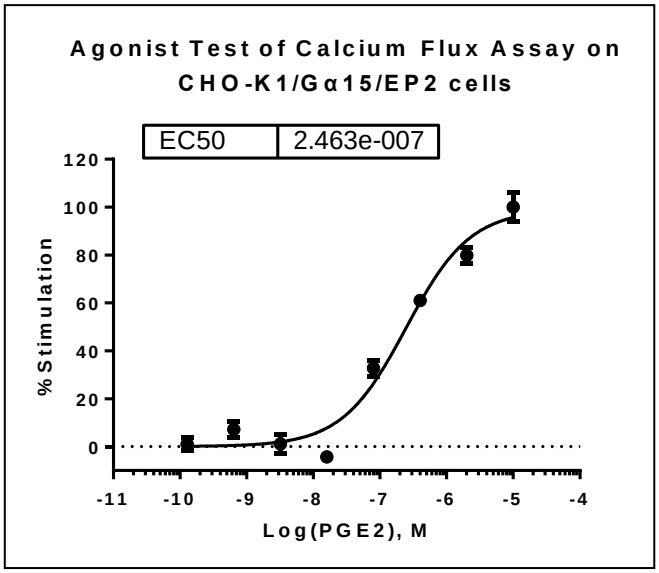

Figure 1. PGE2-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PTGER2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist PGE2. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of PGE2 (Mean ± SD, n = 2). The EC_{50} of PGE2 on this cell was 0.25 μM.

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. Add antibiotic in the following day.

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

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