Human Recombinant EP2 Prostanoid Receptor Stable Cell Line

Technical Manual No. TM0448 **Version 10132010**

I. Introduction

Catalog Number: M00311

Cell Line Name: CHO-K1/EP2/Gα15

Gene Synonyms: PTGER2

Expressed Gene: Genbank Accession Number NM_000956; 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 EP2 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, 400 µg/ml G418, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

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.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by PGE2 in CHO-K1/EP2/Gα15 and CHO-K1 cells

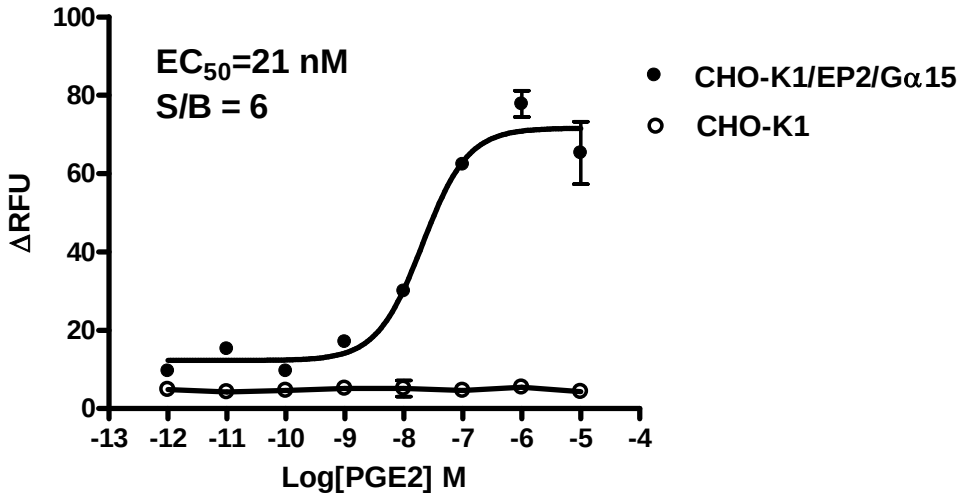

Figure 1. PGE2-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/EP2/Gα15 and CHO-K1. The cells were loaded with Calcium-4 prior to stimulation with an EP2 receptor agonist, PGE2. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PGE2 (Mean ± SD, n = 2). The EC_{50} of PGE2 on EP2 co-expressing with Gα15 in CHO-K1 cells was 21 nM. The S/B of PGE2 on EP2 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. Sugimoto Y, *et al*. (2007) Prostaglandin E receptors. *J. Biol. Chem*. 282(16):11613-11617
2. Regan JW. (2003) EP2 and EP4 prostanoid receptor signaling. *Life Sci.* 74(2-3):143-153.
3. Cimino PJ. (2008) Therapeutic targets in prostaglandin E2 signaling for neurologic disease. *Curr. Med. Chem.* 15(19):1863-1869.

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