Human Recombinant FP Prostanoid Receptor Stable Cell Line

Technical Manual No. TM0393 **Version 10132010**

I. Introduction

Catalog Number: M00155

Cell Line Name: 293/FP/Gα15

Gene Synonyms: FP, C46203, MGC120498, TGFR

Expressed Gene: Genbank Accession Number NM_000959; no expressed tags

Host Cell: 293

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for FP receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 μg/ml Zeocin, 250 μg/ml G418, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Prostaglandin F (2-alpha) is known as a potent luteolytic agent. It is involved in modulating intraocular pressure and smooth muscle contraction in uterus. Its effects on cells are mediated through specific interaction with the Prostanoid receptor FP, which is a 359-amino acid protein. Having 7 putative transmembrane domains, FP resembles the characteristic of G protein coupled receptors. Knockout studies in mice suggest that the interaction of PGF2-alpha with this receptor may initiate parturition in ovarian luteal cells and thereupon induce luteolysis.

§: 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 Cloprostenol in 293/FP/Gα15 and 293 cells

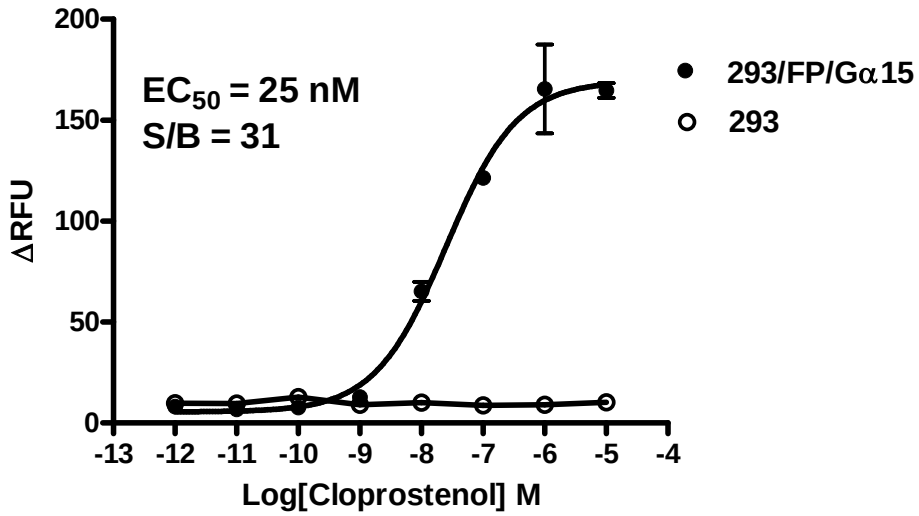

Figure 1. Cloprostenol-induced concentration-dependent stimulation of intracellular calcium mobilization in 293/FP/Gα15 and 293 cells. The cells were loaded with Calcium-4 prior to stimulation with an FP receptor agonist, Cloprostenol. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Cloprostenol (Mean ± SD, n = 2). The EC_{50} of Cloprostenol on FP co-expressing with Gα15 in 293 cells was 25 nM. The S/B of Cloprostenol on FP co-expressing with Gα15 in 293 cells was 31.

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, Zeocin, and G418 to concentrations of 100 µg/ml, 200 µg/ml, and 250 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. Choe, H., M. Farzan, *et al.* (1998). The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J Virol* 72(7): 6113-8.
2. Lake, S., Gullberg, H., Wahlqvist, J., Sjogren, A.-M., Kinhult, A., Lind, P., Hellström-Lindahl E. and Stjernschantz, J. (1994) Cloning of the rat and human prostaglandin F2a receptors and the expression of the rat prostaglandin F2 receptor. *FEBS Lett.*, 355, 317 - 325.

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