Human Recombinant Platelet Activating Factor Receptor Stable Cell Line

Technical Manual No. TM0417 **Version 10132010**

I. Introduction

Catalog Number: M00262

Cell Line Name: CHO-K1/PTAFR

Gene Synonyms: PTAFR, PAFR

Expressed Gene: Genbank Accession Number NM_000952; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for PTAFR 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, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The platelet-activating factor (PAF) receptor (PTAFR) is a G protein coupled receptor that signals through multiple pathways and mediates several cellular responses including cell motility, smooth muscle contraction, and releases of cytokine and leukotriene (Stafforini et al., 2003). In humans, various diseases have been associated with PAF, such as allergic asthma, endotoxic shock, atherosclerosis and psoriasis.

§: 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 PAF in CHO-K1/PTAFR and CHO-K1 cells

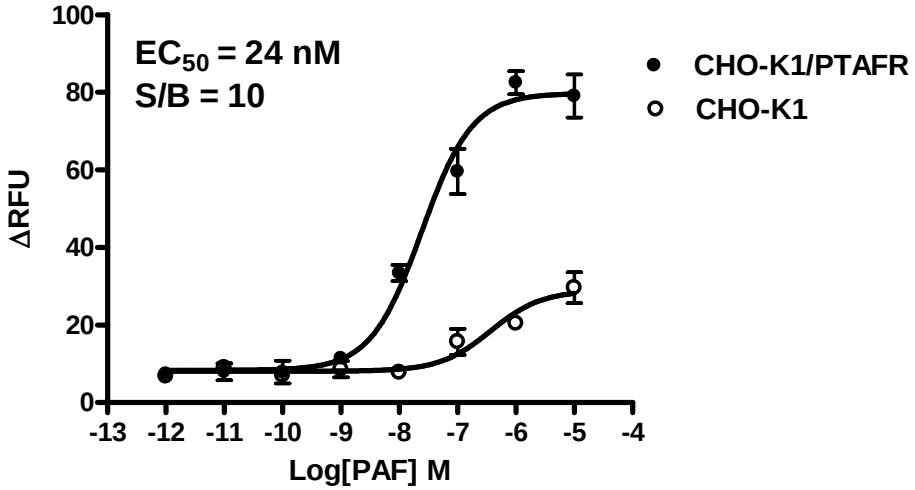

Figure 1. PAF-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PATFR and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a PTAFR receptor agonist, PAF. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PAF (Mean ± SD, n = 2). The EC_{50} of PAF on PTAFR in CHO-K1 cells was 24 nM. The S/B of PAF on PTAFR in CHO-K1 cells was 10.

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 Zeocin to a concentration of 200 µg/ml 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. Dyanand D (2004) Activation of platelet-activating factor receptor-coupled G alpha q leads to stimulation of Src and focal adhesion kinase via two separate pathways in human umbilical vein endothelial cells. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* Vol. 279, No. 5, Issue of January 30, pp. 3497–3508, 2004

2. Denis J. Dupre (2003) Trafficking, ubiquitination, and down-regulation of the human platelet-activating factor receptor .*THE JOURNAL OF BIOLOGICAL CHEMISTRY* Vol. 278, No. 48, Issue of November 28, pp. 48228–48235, 2003

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