Human Recombinant Platelet Activating Factor Receptor Stable Cell Line
Cat. No. M00262 **Version 07282020**

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 (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for PTAFR 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, 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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.

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

III. REPRESENTATIVE DATA

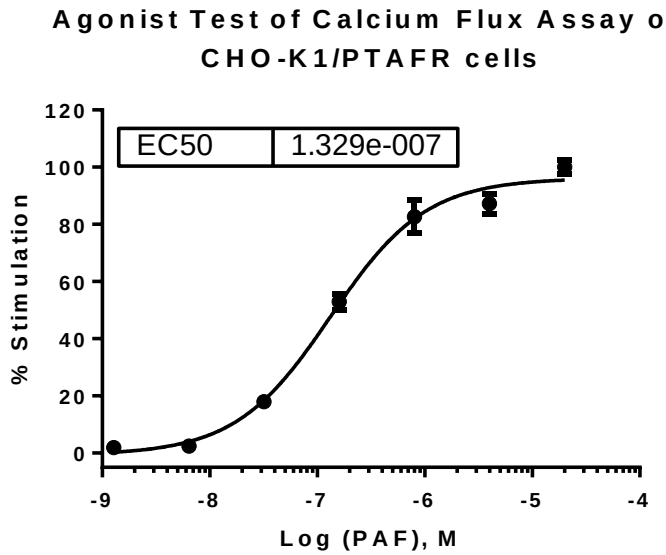

Figure 1. PAF-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PTAFR cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist PAF. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of PAF (Mean ± SD, n = 2). The EC_{50} of PAF on the cells was 0.13 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 RFU and starts at Bottom and goes to Top with a sigmoid shape.

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. 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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