Human Recombinant PAR4 Proteinase-activated Receptor Stable Cell Line

Cat. No. M00206 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00206
Cell Line Name: CHO-K1/PAR4
Gene Synonyms: PAR4, PAR-4, F2RL3
Expressed Gene: Genbank Accession Number NM_003950; 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 PAR4 receptor
Freeze Medium: 45% Ham's F12, 45% FBS, and 10% DMSO
Complete Growth Medium: Ham's F12, 10% FBS
Culture Medium: Ham's F12, 10% FBS, 400 μg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Protease-activated receptor (PAR)-4 is a member of a unique family of GPCRs. The protease-activated receptors (PARs) that are activated by proteolytic cleavage of the N-terminal domain of the receptor reveal a tethered ligand. The PAR family consists of 4 receptors; PAR1 and PAR3 are activated by thrombin, and PAR2 and PAR4 are activated by several serine proteases (Macfarlane et al., 2001). PAR4 is a recently identified low-affinity thrombin receptor that plays a pathophysiological role in many types of tissues including the lung. Mice lacking PAR4 are protected from mesenteric arteriole thrombosis, indicating that PAR4 is a potential target for treatment of thrombosis in humans.

§: 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.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by PAR4 in CHO-K1/PAR4 and CHO-K1 cells

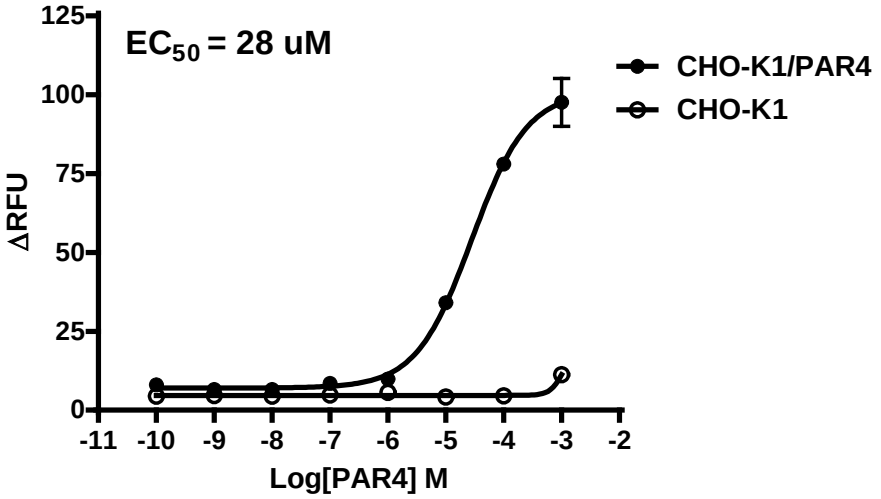

Figure 1. PAR4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PAR4 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a PAR4 receptor agonist, PAR4. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PAR4 (Mean ± SD, n = 2). The EC_{50} of PAR4 on PAR4 in CHO-K1 cells was 28 μM. The S/B of PAR4 on PAR4 in CHO-K1 cells was 14.

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. 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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Mao Y, Jin J, Daniel JL, Kunapuli SP（2009） Regulation of plasmin-induced protease-activated receptor 4 activation in platelets. *Platelets* .2009 May;20(3):191-8.
2. Nieman MT. (2008) Protease-activated receptor 4 uses anionic residues to interact with alpha-thrombin in the absence or presence of protease-activated receptor 1. *Biochemistry*. 2008 Dec 16;47(50):13279-86
3. Ando S, Otani H, Yagi Y, Kawai K, Araki H, Nakamura T, Fukuhara S, Inagaki C. (2007) Protease-activated receptor 4-mediated Ca2+ signaling in mouse lung alveolar epithelial cells. *Life Sci*. 2007 Aug 16;81(10):794-802. Epub 2007 Aug 17

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