Human Recombinant PAR4 Proteinase-activated Receptor Stable Cell Line

Cat. No. M00206 **Version 06232020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for PAR4 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-12 K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12 K (Kaighn's), 10% FBS, 400 μg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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.

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

III. REPRESENTATIVE DATA

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

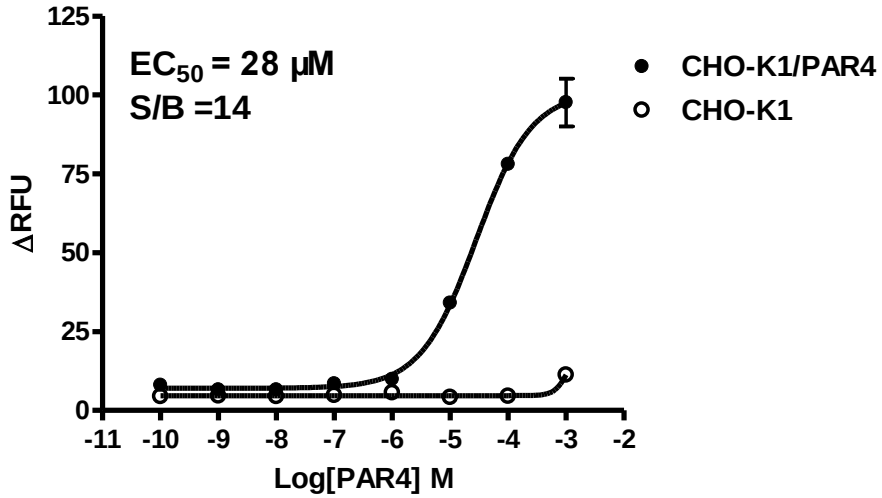

Figure 1: PAR4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PAR4 cells. The cells were loaded with Calcium-4 prior to being stimulated with a PAR4 receptor agonist, PAR4. The intracellular calcium change was measured by FLIPR. The relative fluorescent units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

Note:

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 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 for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO_2.
7. Add antibiotic into the medium on the next day.

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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO_2.

Subcultivation Ratio: 1:3 to 1:8
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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