Human Recombinant PAR2 Protease-activated Receptor Stable Cell Line
Cat. No. M00446 **Version 07312020**

I. Introduction

Catalog Number: M00446

Cell Line Name: CHO-K1/PAR2/Gα15

Gene Synonyms: F2RL1, PAR2

Expressed Gene: Genbank Accession Number NM_005242; no expressed tags

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10^6 per vial)

Stability: More than 16 passages

Application: Functional assay for PAR2 receptor (Calcium flux assay, cAMP 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), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

II. Background

Protease-activated receptors (PAR) are a subfamily of G-protein coupled, seven-transmembrane domain receptors, which are cleaved within the aminoterminal exodomain by certain serine proteases at a specific peptide bond. Trypsin and mast cell tryptase, and more recently, the activated coagulation factors VIIa and Xa, have been identified as serine proteases able to activate mammalian PAR-2. As already indicated, PAR-2 is believed to be involved in inflammation. This role for PAR-2 implies that elastase and cathepsin G would paradoxically display an anti-inflammatory property by disarming PAR-2.

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

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Trypsin in CHO-K1/PAR2/Gα15 cells

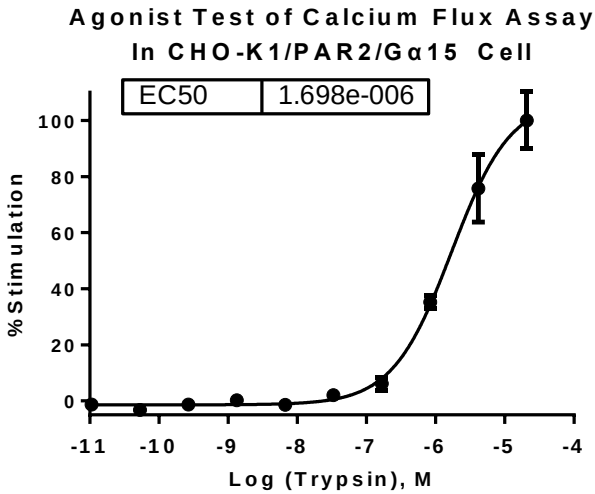

Figure 1. Trypsin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PAR2/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist trypsin. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of trypsin (Mean ± SD, n = 2). The EC_{50} of Trypsin on this cell was 1.7 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 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 and Zeocin to concentrations of 100 μg/ml and 200 μg/ml respectively the following day.

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
Medium Renewal: Every 2 to 3 days

V. References

1. Vergnolle, N. *et al.* (2001) Protease-activated receptors in inflammation, neuronal signaling and pain. *Trends Pharmacol. Sci.* 22(3):146-52
2. Macfarlane, S. R. *et al.* (2001) Proteinase-activated receptors. *Pharmacol Rev.* 53:245-82
3. Cocks, T. M. *et al.* (2001) Protease-activated receptor-2 (PAR2) in the airways. *Pulm. Pharmacol. Ther.* 14(3):183-191
4. Camerer, E. *et al.* (2000) Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proc. Natl. Acad. Sci.* 97(10): 5255–5260.

GenScript USA Inc.
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com

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