

Human Recombinant PAR2 Proteinase-activated Receptor Stable Cell Line

Technical Manual No. TM0568

Version 10132010

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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

Quantity: Two vials of frozen cells (3×10⁶ per vial)

Stability: 16 passages

Application: Functional assay for PAR2 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, 100 µg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Proteinase-activated receptors (PAR) are a subfamily of G-protein coupled, seven-transmembrane domain receptors, which are cleaved within the aminoterminal exodomain by certain serine proteinases 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 proteinases 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.

§: 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 Trypsin in CHO-K1/PAR2/Gα15 and CHO-K1/Gα15 cells

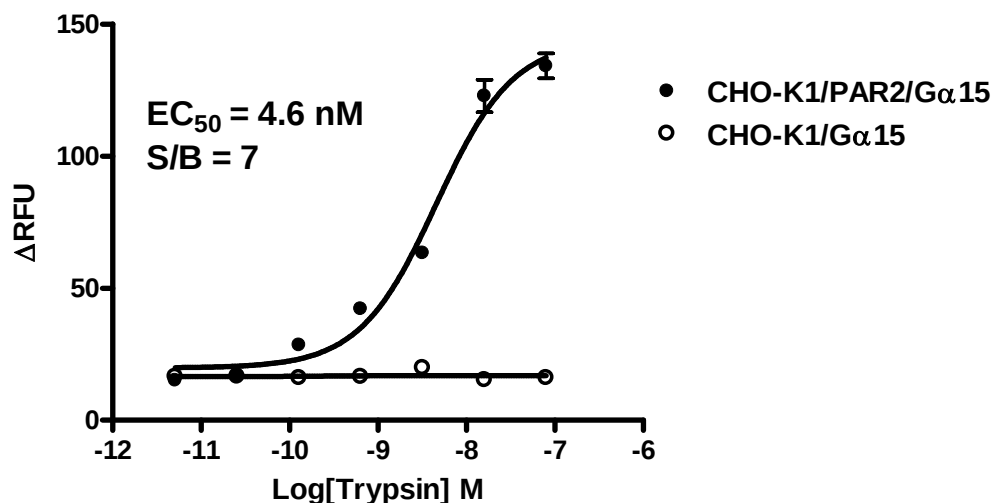


Figure 1. Trypsin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PAR2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a PAR2 receptor agonist, Trypsin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of Trypsin (Mean ± SD, n = 2). The EC₅₀ of Trypsin on PAR2 co-expressing with Gα15 in CHO-K1 cells was 4.6 nM. The S/B of Trypsin on PAR2 co-expressing with Gα15 in CHO-K1 cells was 7.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

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 weekly.

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.

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