Human Recombinant Cysteinyl Leukotriene Receptor 1 Stable Cell Line
Cat. No. M00462 **Version 07312020**

I. INTRODUCTION

Catalog Number: M00462

Cell Line Name: CHO-K1/CysLT1/Gα15

Gene Synonyms: CYSLTR1; CYSLT1; CYSLT1R; CYSLTR; HG55; HMTMF81; MGC46139; LTD4

Expressed Gene: Genbank Accession Number NM_006639; 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 CysLT1 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, 400 μg/ml Geneticin (Cat. #10131-035, Life Technologies), 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

CysLT1 (Cysteinyl leukotriene receptor 1) is previous named as LTD4 receptor (leukotriene D4 receptor). It is a receptor for cysteinyl leukotrienes and has highest affinity to leukotriene D4 (LTD4). The receptor mediates contraction and proliferation of smooth muscle, edema, eosinophil migration and damage to the mucus layer in the lung caused by LTD4. A CysLT1 selective antagonist, montelukast, is used clinically in the treatment of asthma. This response is mediated via a G-protein that activates a phosphatidylinositol-calcium second messenger system. The rank order of affinities for the leukotrienes is LTD4 >> LTE4 = LTC4 >> LTB4.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by LTD4 in CHO-K1/CysLT1/Gα15 cells

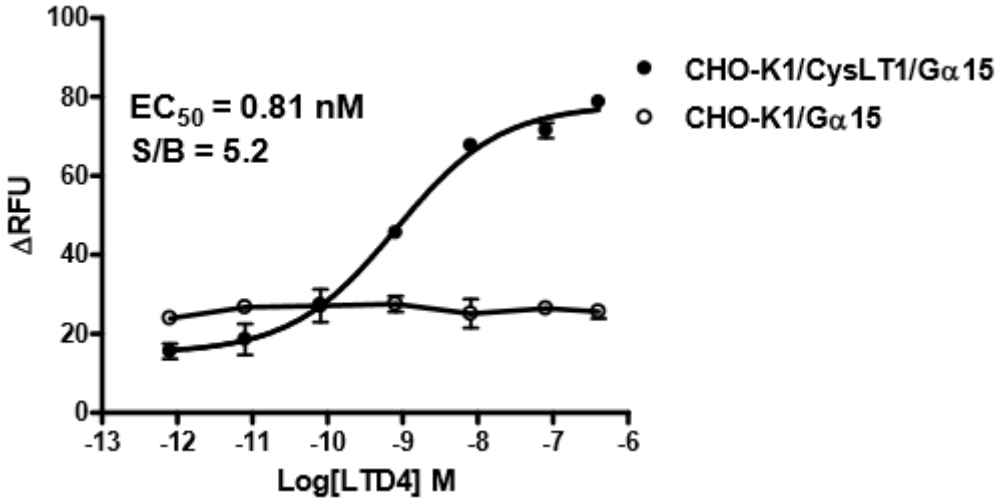

Figure 1. LTD4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CysLT1/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist LTD4. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of LTD4 (Mean ± SD, n = 2). The EC_{50} of LTD4 on this cell was 0.81 nM.

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. Add antibiotic in the following 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 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. Bäck M, et al. (2002).The contraction of the human pulmonary artery by LTC4 is resistant to cysLT1 antagonists and counteracted by prostacyclin release. Adv Exp Med Biol.; 507:315-9.
2. Riccioni G, et al. (2008). Leukotriene modifiers in the treatment of cardiovascular diseases. J Leukoc Biol. Dec; 84(6):1374-8.

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