Human Recombinant Cysteinyl Leukotriene Receptor 1 Stable Cell Line

Technical Manual No. TM0599 **Version 10132010**

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

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for CysLT1 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, 400 μg/ml G418, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

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.

§: 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.

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III. Representative Data

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

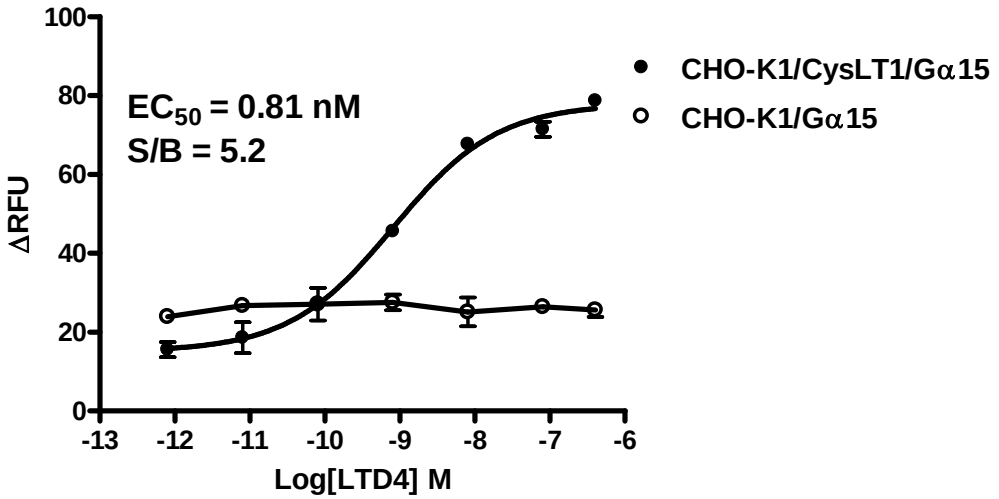

Figure 1. LTD4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CysLT1/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a CysLT1 receptor agonist, LTD4. The intracellular calcium change was measured by FlexStation. The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

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 G418 to concentrations of 100 µg/ml and 400 µg/ml respectively the following day.

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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. 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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