Human Recombinant Leukotriene B4 Receptor Stable Cell Line

Cat. No. M00435 **Version 05272014**

I. INTRODUCTION

Catalog Number: M00435
Cell Line Name: CHO-K1/LTB4/Gα15
Gene Synonyms: LTB4R; BLT1; BLTR; CMKRL1; GPR16; LTB4R1; LTBR1; P2RY7; P2Y7
Expressed Gene: Genbank Accession Number NM_181657; no expressed tags
Host Cell: CHO-K1/$G_{\alpha 15}$
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for LTB4 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 HygromycinB
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The dihydroxy-leukotriene, leukotriene B_4 (LTB_4) is a member of the G protein-coupled receptor (GPCR) family, in a subfamily of GPCRs that includes receptors for chemokines and other chemotactic factors. Recently, a second, lower affinity receptor for LTB4 (BLT2) has also been cloned, with broader ligand specificity for various eicosanoids. LTB4 stimulates neutrophil chemotaxis and secretion but may also affect immunomodulation, contraction of certain smooth muscles via an indirect mechanism and activation of the nuclear transcription factor PPARα (peroxisome proliferation activated receptor alpha). Chemotaxis, the principal effects of LTB_4 and related dihydroxy-acids on leukocytes, occurs via activation of BLT_1 receptors.

§: 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 LTB4 in CHO-K1/LTB4/$G_{\alpha 15}$ and CHO-K1/$G_{\alpha 15}$ cells

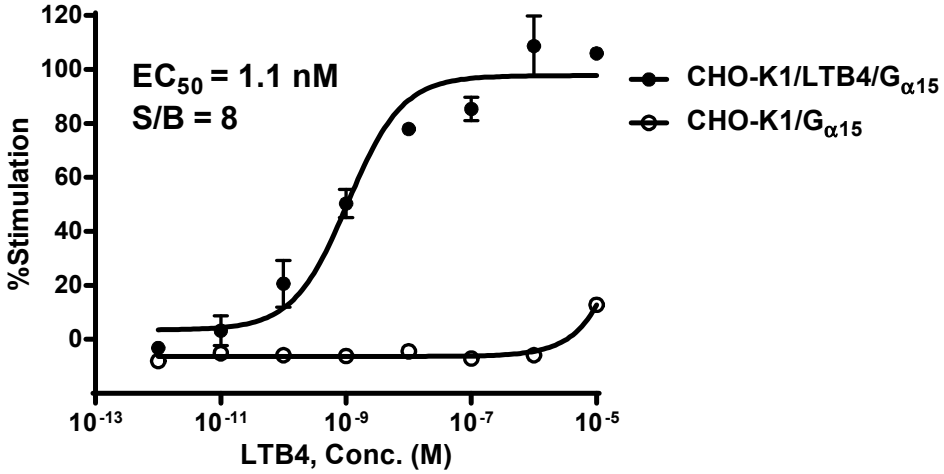

Figure 1. LTB4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/LTB4/$G_{\alpha 15}$ and CHO-K1/$G_{\alpha 15}$ cells. The cells were loaded with Calcium-4 prior to stimulation with an LTB4 receptor agonist, LTB4. The intracellular calcium change was measured by FlexStation3. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of LTB4 (Mean ± SD, n = 2). The EC_{50} of LTB4 on LTB4 co-expressing with $G_{\alpha 15}$ in CHO-K1 cells was 1.1 nM. The S/B of LTB4 on LTB4 co-expressing with $G_{\alpha 15}$ in CHO-K1 cells was 8.

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.

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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.

V. REFERENCES

1. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., *et al.* (1980) Leukotriene B4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature*. 286. 264-265
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