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/Gα15  
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.
III. Representative Data
Concentration-dependent stimulation of intracellular calcium mobilization by LTB4 in CHO-K1/LTB4/Gα15 and CHO-K1/Gα15 cells

![Graph showing concentration-dependent stimulation of intracellular calcium mobilization by LTB4 in CHO-K1/LTB4/Gα15 and CHO-K1/Gα15 cells.]

**Figure 1.** LTB4-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/LTB4/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an LTB4 receptor agonist, LTB4. The intracellular calcium change was measured by FlexStation. 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 Gα15 in CHO-K1 cells was 1.1 nM. The S/B of LTB4 on LTB4 co-expressing with Gα15 in CHO-K1 cells was 8.

Notes:
1. EC$_{50}$ value is calculated with four parameter logistic equation:
   \[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1+10^{(\text{LogEC}_{50} - X) \times \text{HillSlope}})} \]
   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 G418 to concentrations of 100 μg/ml and 400 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

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