Human Recombinant EDG4 Lysophospholipid Receptor Stable Cell Line

Cat. No. M00463 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00463

Cell Line Name: RH7777/EDG4

Gene Synonyms: LPAR2, EDG4, EDG-4, LPA2

Expressed Gene: GenBank Accession Number NM_004720; no expressed tags

Host Cell: RH7777

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

Stability: 16 passages

Applications: Functional assays for EDG4 Receptor

Freeze Medium: 45% culture medium, 45%FBS, and 10% DMSO

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 100 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Lysophosphatidic acid (LPA), a bioactive lipid produced by several cell types including postmitotic neurons and activated platelets, is thought to be involved in various biological processes, including brain development. Three cognate G-protein coupled receptors encoded by lpa(1)/lp(A1)/Edg-2/Gpcr26, lpa(2)/lp(A2)/Edg-4, and lpa(3)/lp(A3)/Edg-7 mediate the cellular effects of LPA. However, many LPA-induced responses, including phospholipase C activation, Ca^{2+} mobilization, adenylyl cyclase activation, proliferation, JNK activation, AKT activation, and stress fiber formation, were absent or severely reduced in embryonic fibroblasts derived from lpa(1)(-/-) lpa(2)(-/-) mice. The lysophosphatidic acid receptor LPA2 or endothelial differentiation, G-protein coupled receptor 4(EDG-4) is expressed most abundantly in testes and peripheral blood leukocytes. It is reported to be a distinctive functional marker for ovarian carcinoma.

§: 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 C18:1 LPA in RH7777/EDG4 and RH7777 cells

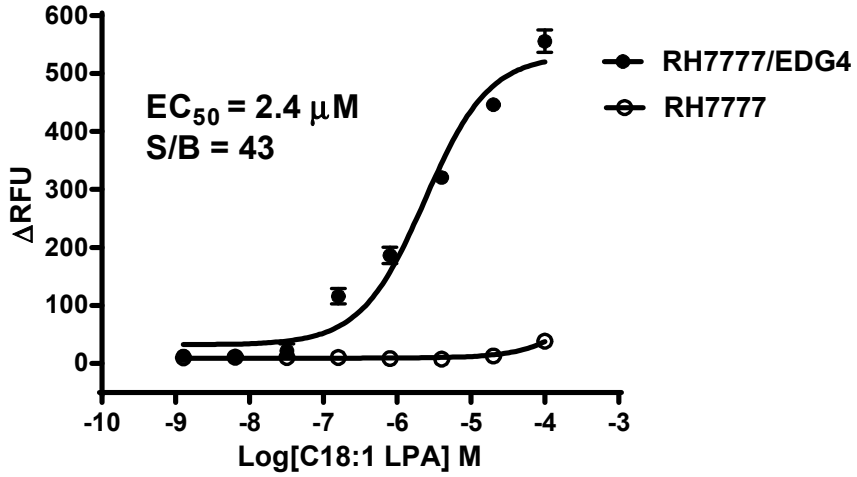

Figure 1. C18:1 LPA-induced concentration-dependent stimulation of intracellular calcium mobilization in RH7777/EDG4 and RH7777 cells. The cells were loaded with Calcium-4 prior to stimulation with an EDG4 receptor agonist, C18:1 LPA. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of C18:1 LPA (Mean ± SD, n = 2). The EC_{50} of C18:1 LPA on EDG4 in RH7777 cells was 2.4 μM. The S/B of C18:1 LPA on EDG4 in RH7777 cells was 43.

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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Simpson PB, *et al.* (2002), Native and recombinant human Edg4 receptor-mediated Ca(2+) signalling. *Assay Drug Dev Technol.* 1(1 Pt 1):31-40.
2. Contos JJ, *et al.* (2002). Characterization of lpa(2) (Edg4) and lpa(1)/lpa(2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to lpa(2). *Mol Cell Biol.* 22(19):6921-9.
3. Hama K, *et al.* (2002).Lysophosphatidic acid (LPA) receptors are activated differentially by biological fluids: possible role of LPA-binding proteins in activation of LPA receptors. *FEBS Lett.* 523(1-3):187-92.

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