Human Recombinant ADRB1 Adrenoceptors Stable Cell Line

Cat. No. M00269 **Version 05222014**

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I. INTRODUCTION

Catalog Number: M00269

Cell Line Name: CHO-K1/ADRB1/Gα15

Gene Synonyms: β1-adrenoceptor, ADRB1

Expressed Gene: Genbank Accession Number NM_000684; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for ADRB1 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, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The ß-adrenergic receptors are linked to G proteins. The ß-receptor has three known subtypes. Beta-1 receptors primarily regulate myocardial tissue and affect the rate of contraction via impulse conduction. Beta-2 receptors regulate smooth muscle tone and influence vascular and bronchiolar relaxation. Beta-3 receptors are less well studied but are thought to primarily affect lypolysis and may have effects on cardiac inotropy (Greene Shepherd，2006). In the human heart, beta (1)- and beta (2)AR are the most powerful physiologic mechanism to acutely increase cardiac performance. Changes in betaAR play an important role in chronic heart failure (CHF). Thus, due to increased sympathetic activity in CHF, betaAR are chronically (over) stimulated, and that results in beta (1) AR desensitization and alterations of down-stream mechanisms (Brodde OE，2006).

§: 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 Epinephrine in CHO-K1/ADRB1/Gα15 and CHO-K1 cells

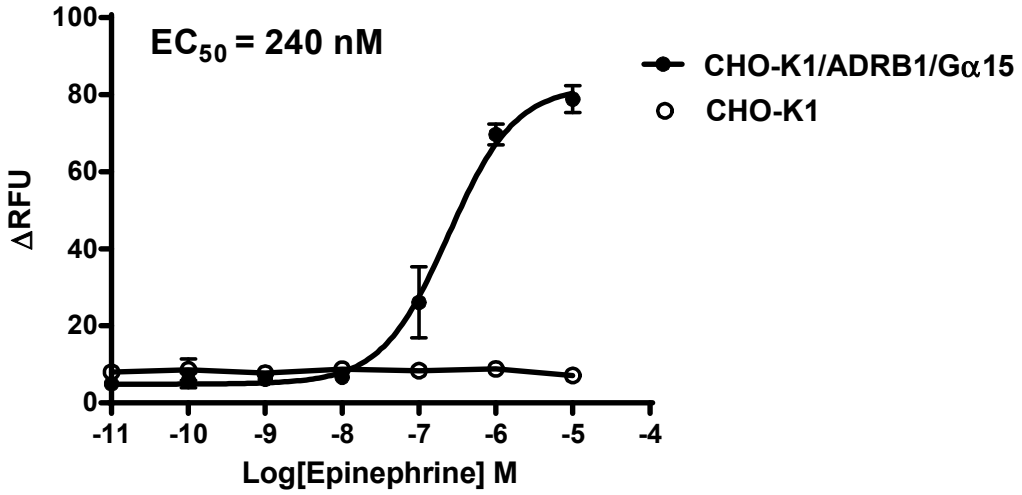

Figure Epinephrine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRB1/Gα15 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a ADRB1 receptor agonist, Epinephrine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Epinephrine (Mean ± SD, n = 2). The EC50 of Epinephrine on ADRB1 co-expressing with Gα15 in CHO-K1 cells was 240 nM. The S/B of Epinephrine on ADRB1 in CHO-K1 cells was 17.

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 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. In the following day, replace the cells with fresh medium contains antibiotic.

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. Greene Shepherd**.** Treatment of poisoning caused by ß-adrenergic and calcium-channel blockers Kukkonen JP et al. (2006) Functions of the orexinergic/hypocretinergic system. *American Journal of Health-System Pharmacy*, Vol. 63, Issue 19, 1828-1835

2. Brodde OE, Bruck H, Leineweber K. (2006)Cardiac adrenoceptors: physiological and pathophysiological relevance .*J Pharmacol Sci.* 2006;100(5):323-37. Epub 2006 Apr 13.

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