Human Recombinant ADRA2B Adrenoceptors Stable Cell Line

Cat. No. M00454 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00454

Cell Line Name: CHO-K1/ADRA2B/Gqi5

Gene Synonyms: ADRA2B, ADRA2L1, ADRA2RL1, ADRARL1, ALPHA2BAR

Expressed Gene: Genbank Accession Number NM_000682; no expressed tags

Host Cell: CHO-K1/Gqi5

Quantity: 2 vial (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for ADRA2B receptor

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

Complete Culture 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

Adrenoceptors are 7-transmembrane receptors which mediate the central and peripheral actions of the neurotransmitter, noradrenaline (norepinephrine), and the hormone and neurotransmitter, adrenaline (epinephrine). Based on both pharmacological and molecular evidence, they are divided into three major types - α1, α2, and β. The α2 adrenoceptors include 3 subtypes - α2A, α2B, and α2C – each of which couples to Gi/o protein primarily.

The α2B adrenoceptors (ADRA2B) is expressed in the kidney, liver, brain, lung, heart, and skeletal muscle. The development of α2B and α2C knock-out mice has shown that these two subtypes are not involved in the central hypotensive response to α2 agonists.

§: 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 UK14304 in CHO-K1/ADRA2B/Gqi5 and CHO-K1/Gqi5 cells

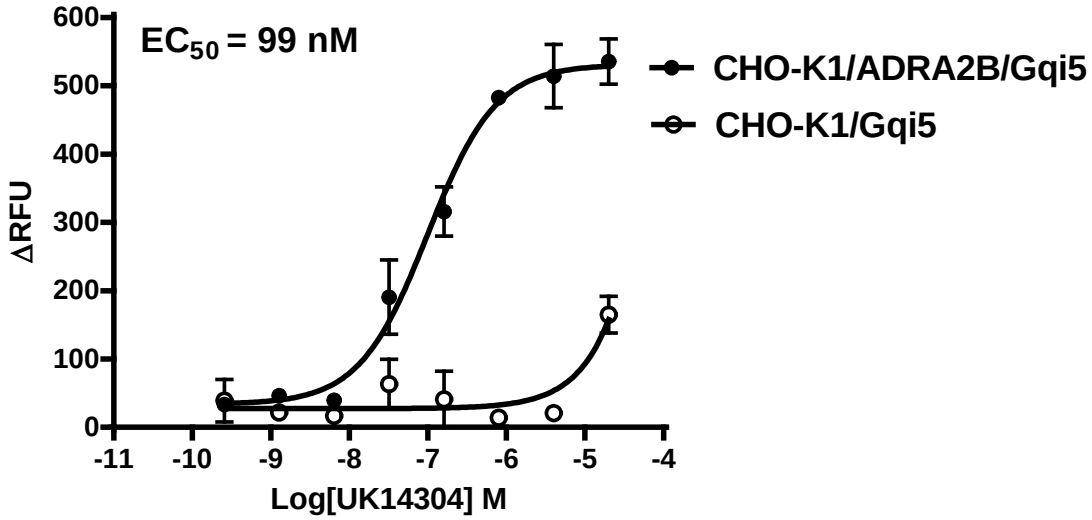

Figure UK14304-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRA2B/Gqi5 and CHO-K1/Gqi5 cells. The cells were loaded with Calcium-4 prior to stimulation with a ADRA2B receptor agonist, UK14304. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of UK14304 (Mean ± SD, n = 2). The EC_{50} of UK14304 on ADRA2B co-expressing with Gqi5 in CHO-K1 cells was 99 nM. The S/B of UK14304 on ADRA2B co-expressing with Gqi5 in CHO-K1 cells was 16.

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. 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. Handy DE *et al.* (1993) Diverse tissue expression of rat alpha 2-adrenergic receptor genes. *Hypertension.* 21(6 Pt 1):861-5.

2. Makaritsis KP *et al.* (1999) Role of the alpha2B-adrenergic receptor in the development of salt-induced hypertension. *Hypertension.* 33(1):14-7.

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