Human Recombinant ADRA2A Adrenoceptors Stable Cell Line

Cat. No. M00281 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00281

Cell Line Name: CHO-K1/ADRA2A/Gα15

Gene Synonyms: ADRA2A; ADRA2; ADRA2R; ADRAR; ALPHA2AAR; ZNF32

Expressed Gene: Genbank Accession Number NM_000681; 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 ADRA2A 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, 200 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The adrenoceptors ADRA2A is expressed in the brain, spleen, kidney, aorta, lung, skeletal muscle, heart and liver. ADRA2A -adrenoceptor knockout mice exhibit disruption of presynaptic inhibition of noradrenaline release at high stimulation frequencies. ADRA2A receptor is the principal autoreceptor in the presynaptic feedback loop regulating noradrenaline release. However, another α2 autoreceptor is also present.

§: 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/ADRA2A/ Gα15 and CHO-K1/Gα15 cells

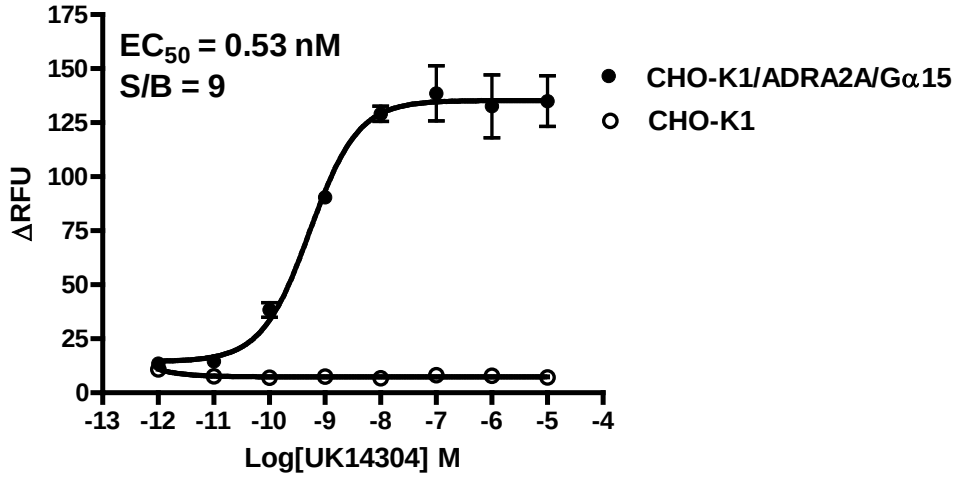

Figure. UK140304-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRA2A/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADRA2C receptor agonist, UK140304. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of UK140304 (Mean ± SD, n = 2). The EC_{50} of UK140304 on ADRA2A co-expressing with Gα15 in CHO-K1 cells was 0.53 nM. The S/B of ACTH on ADRA2A co-expressing with Gα15 in CHO-K1 cells was 9.

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. Hein L, Altman JD, Kobilka BK. (1999) Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. *Nature.* 402(6758):181-4.

2. Perälä M, (1992) Differential expression of two alpha 2-adrenergic receptor subtype mRNAs in human tissues. *Brain Res Mol Brain Res.* 16(1-2):57-63.

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