

Human Recombinant ADRA2C Adrenoceptors Stable Cell Line**Cat. No. M00252****Version 05282014**

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I. INTRODUCTION

Catalog Number: M00252

Cell Line Name: CHO-K1/ADRA2C/Gα15

Gene Synonyms: ADRA2C; ADRA2L2; ADRA2RL2; ADRARL2; ALPHA2CAR

Expressed Gene: Genbank Accession Number NM_000683; 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 ADRA2C 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 Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The adrenoceptors ADRA2C is expressed in the brain, kidney, aorta, lung, skeletal muscle, heart and spleen.

ADRA2C knockout mice exhibited disrupts presynaptic inhibition of noradrenaline release at low stimulation frequencies.

§: 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.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by UK14304 in CHO-K1/ADRA2C/Gα15 and CHO-K1/Gα15 cells

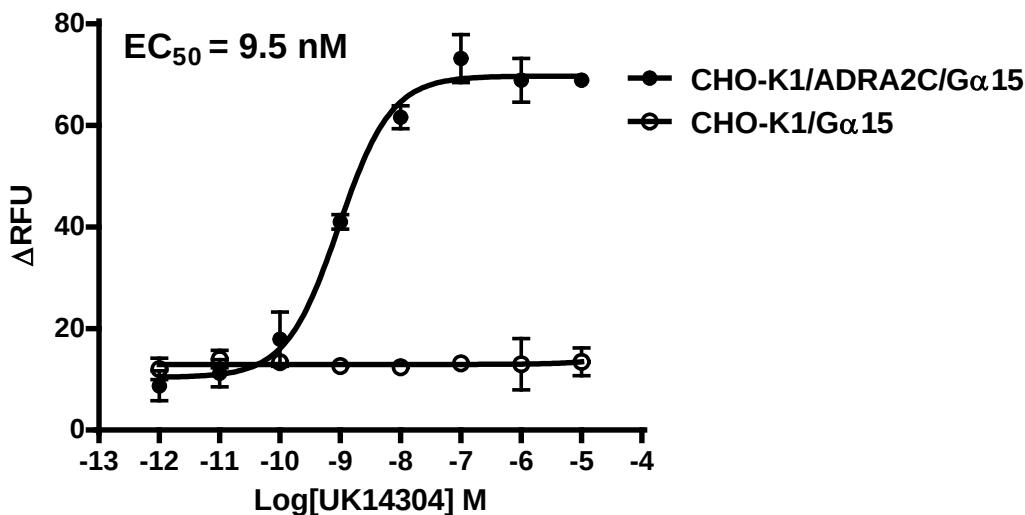


Figure UK14304-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRA2C/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADRA2C receptor agonist, UK14304. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of UK14304 (Mean ± SD, n = 2). The EC₅₀ of UK14304 on ADRA2C co-expressing with Gα15 in CHO-K1 cells was 9.5 nM. The S/B of ACTH on ADRA2C co-expressing with Gα15 in CHO-K1 cells was 7.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

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.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.

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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 %CO₂.
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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Eason MG, Liggett SB. (1993) Human alpha 2-adrenergic receptor subtype distribution: widespread and subtype-selective expression of alpha 2C10, alpha 2C4, and alpha 2C2 mRNA in multiple tissues. *Mol Pharmacol.* 44(1):70-5.
2. Hein L, Altman JD, Kobilka BK. (1999) Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. *Nature.* 402(6758):181-4.

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