Human Recombinant ADRA1D Adrenoceptors Stable Cell Line

Cat. No. M00340 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00340
Cell Line Name: CHO-K1/ADRA1D
Gene Synonyms: ADRA1D; ADRA1; ADRA1A; ADRA1R; ALPHA1; DAR; dJ779E11.2
Expressed Gene: GenBank Accession Number NM_000678; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Applications: Functional assays for ADRA1D receptors
Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO
Complete Growth Medium: Ham's F12, 10% FBS
Culture Medium: Ham's F12, 10% FBS, 400 μg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The α_1-adrenergic receptor (AR) family consists of three closely related gene products (α_{1A}, α_{1B}, and α_{1D}). They mediate the actions of norepinephrine (NE) and epinephrine in sympathetically innervated tissues and brain. α_1-ARs belong to the G protein-coupled receptor family and consist of single polypeptide chains that are predicted to form seven transmembrane spanning domains. With similar pharmacological and signaling properties, α_1-AR subtypes act through $G_{q/11}$ proteins to activate phospholipase C, increase inositol 1,4,5-trisphosphate production, and increase intracellular Ca^{2+}.

§: 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/ADRA1D cells

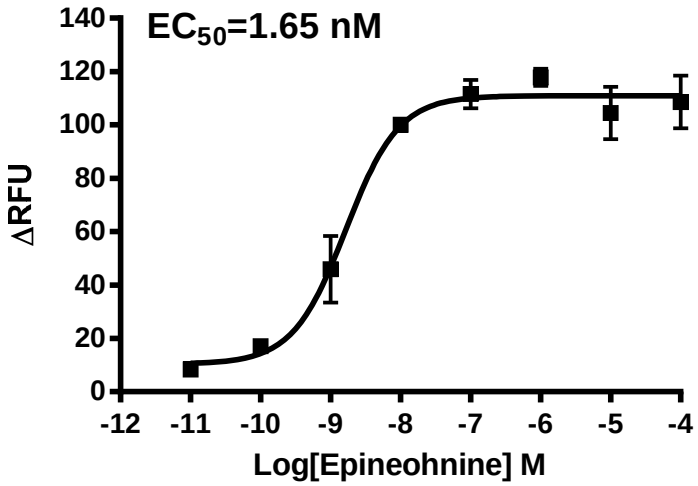

Figure 1. Epinephrine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRA1D cells. The cells were loaded with Calcium-4 prior to stimulation with a ADRA1D receptor agonist, Epinephrine. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Epinephrine (Mean ± SD, n = 2). The EC_{50} of Epinephrine on ADRA1D in CHO-K1 cells was 1.65 nM. The S/B of Epinephrine on ADRA1D in CHO-K1 cells was 11.

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.

Subculturing 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. Vicentic, A., Robeva, A., Rogge, G., Uberti, M. and Minneman, K.P.(2002) Biochemistry and Pharmacology of Epitope-Tagged α_1--Adrenergic Receptor Subtypes *J. Pharmacol. Exp. Ther., 302: 58-65*
2. Zhong H and Minneman KP (1999a) α_1-Adrenoceptor subtypes. *Eur J Pharmacol 375:261–276.*
3. Ruffolo RR Jr, Stadel JM, and Hieble JP (1994) α1-Adrenoceptors: recent developments.*Med Res Rev 14:229–270.*

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