

Human Recombinant ADRB1 Adrenoceptors Stable Cell Line

Technical Manual No. TM0418

Version 10132010

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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 (3×10⁶ 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 β-adrenergic receptors are linked to G proteins. The β-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 lipolysis 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).

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Epinephrine in CHO-K1/ADRB1/Gα15 and CHO-K1 cells

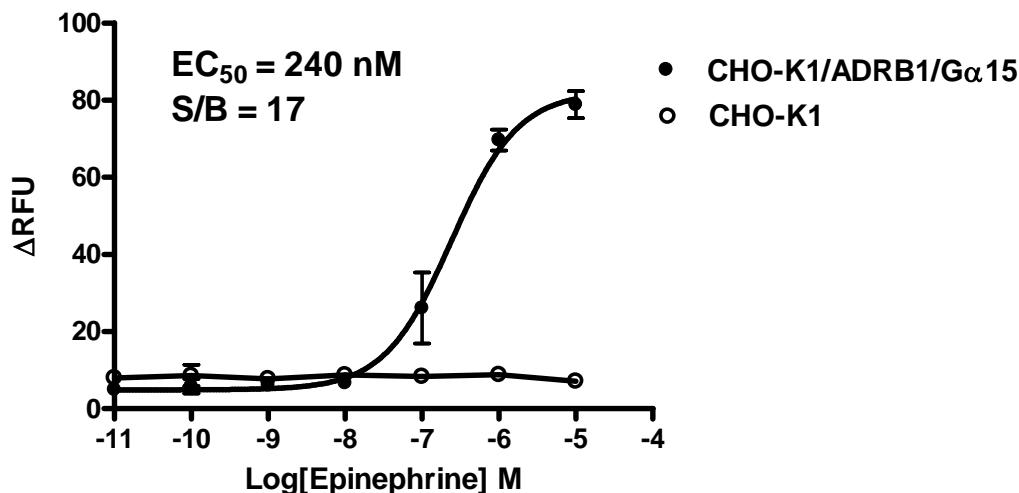


Figure 1. Epinephrine-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRB1/Gα15 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a ADRB1 receptor agonist, Epinephrine. The intracellular calcium change was measured by FlexStation. 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:

- 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 discard the medium.
- Resuspend the cells in complete growth medium.
- Add 10 ml of the cell suspension in a 10 cm dish.
- Add Hygromycin B and Zeocin to concentrations of 100 µg/ml and 200 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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 β -adrenergic and calcium-channel blockers Kukkonen JP et al. (2006) Functions of the orexinergic/hypocretinerbic 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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