Human Recombinant ADRB2 Adrenoceptors Stable Cell Line

Technical Manual No. TM0504 **Version 10132010**

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I. Introduction

Catalog Number: M00308

Cell Line Name: CHO-K1/ADRB2/Gα15

Gene Synonyms: BAR; B2AR; ADRBR; ADRB2R; BETA2AR; ADRB2

Expressed Gene: Genbank Accession Number NM_000024; 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 ADRB2 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

β-Adrenergic receptors (β-ARs) are members of the superfamily G-protein-coupled receptors that are stimulated by naturally occurring catecholamines, epinephrine, and norepinephrine. As part of the sympathetic nervous system, β-ARs have been shown to have important roles in cardiovascular, respiratory, metabolic, central nervous system, and reproductive functions. Three distinct β-AR subtypes have been identified (β_1-AR, β_2-AR, and β_3-AR). All three of these β-AR subtypes are believed to signal by coupling to the stimulatory G-protein Gs_α which leads to the activation of adenylyl cyclase and accumulation of the second messenger cAMP. β_1-ARs mediate cardiac stimulation, β_2-ARs mediate smooth muscle relaxation in the peripheral vasculature and respiratory system, and β_3-AR has been shown to have important roles in adipose tissue and gastrointestinal tract. In studies using subtype-selective agonists and antagonists in the human heart, β_2-AR stimulation leads to the activation of adenylyl cyclase and contributes to both inotropic and chronotropic responses.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by Isoproterenol in CHO-K1/ADRB2/Gα15 and CHO-K1/Gα15 cells

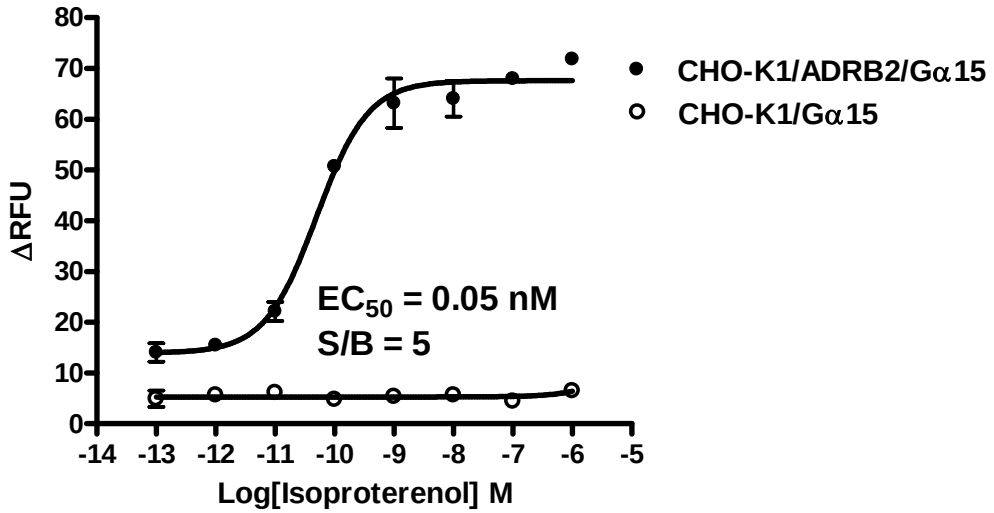

Figure 1. Isoproterenol-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADRB2/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a ADRB2 receptor agonist, Isoproterenol. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Isoproterenol (Mean ± SD, n = 2). The EC_{50} of Isoproterenol on ADRB2 co-expressing with Gα15 in CHO-K1 cells was 0.05 nM. The S/B of Isoproterenol on ADRB2 co-expressing with Gα15 in CHO-K1 cells was 5.

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 the 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 for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and Zeocin to concentrations of 100 µg/ml and 200 µg/ml respectively the following day

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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 a 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 for 5min, and discard the medium.
5. Resuspend the cells in culture medium 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

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