Human Recombinant Adenosine A3 Receptor Stable Cell Line
Cat. No. M00464 **Version 12182015**

I. INTRODUCTION

Catalog Number: M00464

Cell Line Name: CHO-K1/ADORA3/$G_{\alpha 15}$

Gene Synonyms: ADORA3, A3AR, AD026, RP11-552M11.7, bA552M11.5

Expressed Gene: GenBank Accession Number NM_000677; no expressed tags

Host Cell: CHO-K1/ $G_{\alpha 15}$

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Applications: Functional assays for ADORA3 receptor

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, 3 μg/mL puromycin, and 100 μg/mL Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four G-protein coupled adrenoceptors, A1, A2A, A2B and A3, is responsible for these effects. A3, which couples to $G_{i/o}$, is expressed in a wide range of human tissues, but most predominantly in the lung and liver. Recent animal model studies have shown that A3 receptors play important roles in brain ischemia, immunosuppresion, and bronchospasm. A3 receptor agonists and/or agonists may have important clinical value in the treatment of asthma and inflammation. Mice lacking A3 receptors display reduced mast cell degranulation and bronchoconstriction in response to adenosine.

§: 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 NECA in CHO-K1/ADORA3/ $G_{\alpha 15}$ and CHO-K1/$G_{\alpha 15}$ cells

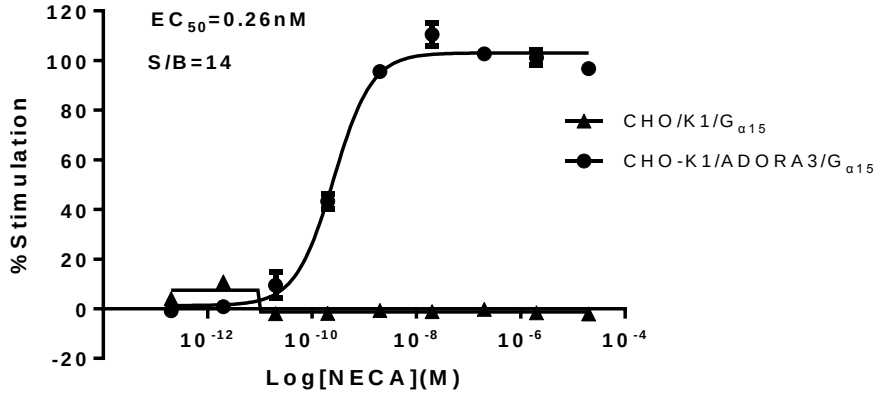

Figure: NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA3/$G_{\alpha 15}$ and CHO-K1/$G_{\alpha 15}$ cells. The cells were loaded with Calcium-4 prior to stimulation with a ADORA3 receptor agonist NECA. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NECA (Mean±SD, n=2). The EC_{50} of NECA on ADORA3 in CHO-K1 cells was 0.26 nM. The S/B of NECA on ADORA3 in CHO-K1/$G_{\alpha 15}$ cells was 14.

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. Add antibiotic in the following day.

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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. Baraldi *et al.* (2000). A (3) adenosine receptor ligands: history and perspectives. *Med Res Rev* 20:103-128.
2. Schepp *et al.* (2008) Bench-to-bedside review: adenosine receptors – promising targets in acute lung injury? *Crit Care* 12:226.
3. Fredholm BB *et al.* (2000) Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch Pharmacol.* 362(4-5):364-74.
4. Tilley SL, *et al.* (2000) Identification of A3 receptor- and mast cell-dependent and -independent components of adenosine-mediated airway responsiveness in mice. *J Immunol.* 171(1):331-7.
5. Zhong H, *et al.* (2000) Activation of murine lung mast cells by the adenosine A3 receptor. *J Immunol.* 171(1):338-45.

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