

Human Recombinant Adenosine A2B Receptor Stable Cell Line**Cat. No. M00329****Version 05282014**

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I. INTRODUCTION

Catalog Number: M00329

Cell Line Name: CHO-K1/ADORA2B/Gα15

Gene Synonyms: ADORA2B;

Expressed Gene: Genbank Accession Number NM_000676; 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 ADORA2B 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 adenosine receptors ADORA2B is G_s and G_q/G_{11} -coupled GPCR expressed in the large intestine, cecum, and urinary bladder. ADORA2B receptor mediates relaxation to adenosine in human small coronary arteries which is independent of NO but depends in part on a K^+ -sensitive mechanism. Pharmacological or molecular biologic activation of ADORA2B receptor may prevent glomerular remodeling associated with glomerulosclerosis, renal disease, and abnormal growth associated with hypertension and diabetes.

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§: 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. arthritis*, *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 NECA in CHO-K1/ADORA2B/Gα15 and CHO-K1/Gα15 cells

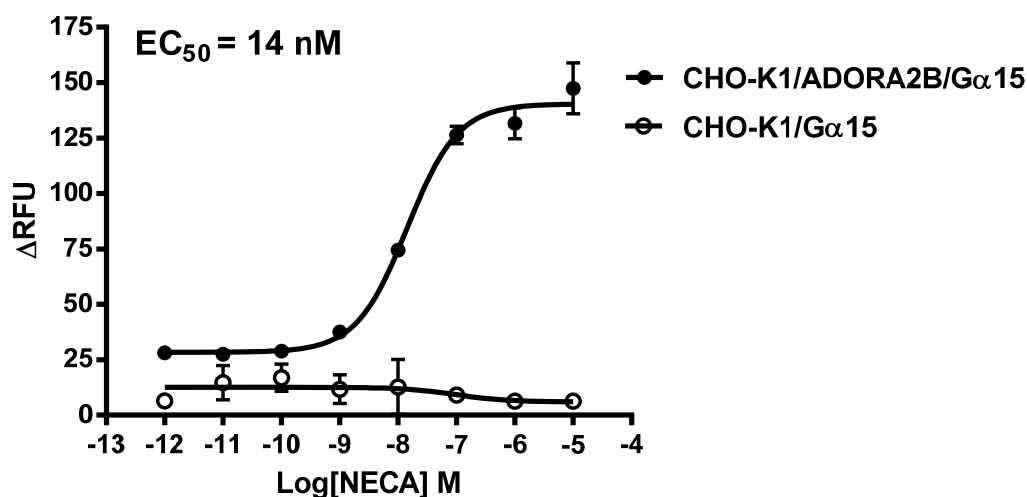


Figure 1. NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA2B/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADORA2B receptor agonist, NECA. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NECA (Mean ± SD, n = 2). The EC₅₀ of NECA on ADORA2B co-expressing with Gα15 in CHO-K1 cells was 14 nM. The S/B of NECA on ADORA2B co-expressing with Gα15 in CHO-K1 cells was 5.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

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

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.

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.

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 %CO₂.

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

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Stehle JH *et al.* (1992) Molecular cloning and expression of the cDNA for a novel A2-adenosine receptor subtype. *Mol Endocrinol.* 6(3):384-93.
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3. Kemp BK *et al.* (1999) Adenosine mediates relaxation of human small resistance-like coronary arteries via A2B receptors. *Br J Pharmacol.* 126(8):1796-800.
4. Dubey RK *et al.* (2005) Adenosine inhibits PDGF-induced growth of human glomerular mesangial cells via A(2B) receptors. *Hypertension.* 46(3):628-34. Epub 2005 Aug 15.

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