Human Recombinant Adenosine A1 Receptor Stable Cell Line
Cat. No. M00324 **Version 05282014**

I. INTRODUCTION

Catalog Number: M00324
Cell Line Name: CHO-K1/ADORA1
Gene Synonyms: ADORA1
Expressed Gene: Genbank Accession Number NM_000674; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for ADORA1 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
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery.

II. BACKGROUND

ADORA1 is a receptor for adenosine. It is demonstrated that the ADORA1 receptor is ubiquitous throughout the entire body. Activation of ADORA1 elicits an inhibition of adenylate cyclase and therefore a decrease in the cAMP concentration. ADORA1 receptors are implicated in sleep promotion by inhibiting wake promoting cholinergic neurons in the basal forebrain. Adenosine antagonists are widely used in neonatal medicine.

§: 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 NECA in CHO-K1/ADORA1/Gα15 and CHO-K1/Gα15 cells

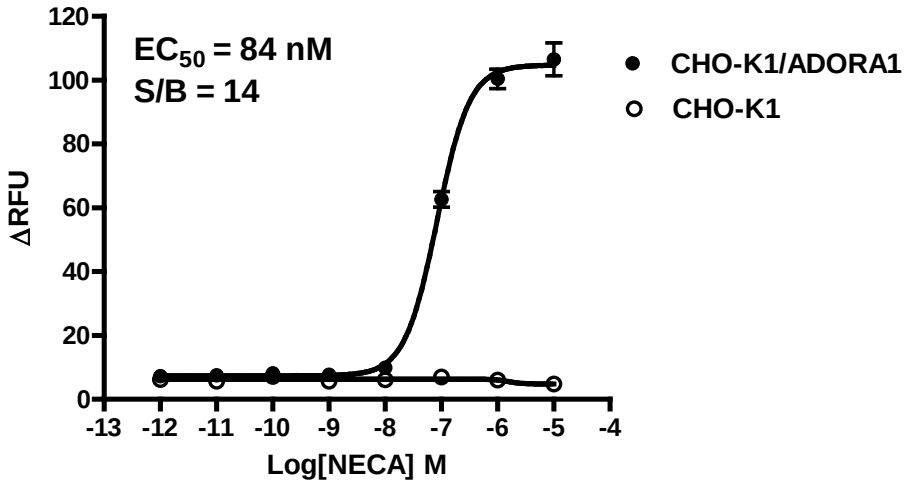

Figure 1. NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a ADORA1 receptor agonist, NECA. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NECA (Mean ± SD, n = 2). The EC_{50} of NECA on ADORA1 in CHO-K1 cells was 84 nM. The S/B of NECA on ADORA1 in CHO-K1 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. 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. Elmenhorst, D., Meyer, PT., Winz, OH., *et al.* (2007) Sleep deprivation increases A1 adenosine receptor binding in the human brain: a positron emission tomography study. *J. Neurosci.* 27 (9): 2410–2415
2. Fredholm, BB, IJzerman, AP., Jacobson, KA., *et al.* (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53 (4): 527–552.

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