Mouse Recombinant Adenosine A2A Receptor Stable Cell Line
Cat. No. M00575 **Version 08152016**

I. INTRODUCTION

Catalog Number: M00575
Cell Line Name: CHO-K1/mouse ADORA2A
Gene Synonyms: RDC8; A2-AR; adenosine receptor A2a
Expressed Gene: Genbank Accession Number NM_009630.3; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (1×10^6 per vial)
Stability: 16 passages
Application: Functional assay for mouse ADORA2A receptor
Freeze Medium: 95% complete growth medium, 5% DMSO
Complete Growth Medium: F-12K, 10% FBS
Culture Medium: F-12K, 10% FBS, 450 μg/ml Zeocin
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The adenosine receptors ADORA2A are Gs-coupled GPCRs expressed in the thymus gland, heart, lung, kidney, brain, platelets, spleen and leukocytes. ADORA2A down-regulates chemokine receptor function and inhibits platelet aggregation. ADORA2A antagonists may be useful as therapy for Parkinson's disease.

§: 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

This cell based assay is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). It is a competitive immunoassay that uses cAMP labeled with the d2 acceptor flourophore and an anti-cAMP monoclonal AB labeled with Europium Cryptate. The FRET signal decreases as cAMP concentration rises.

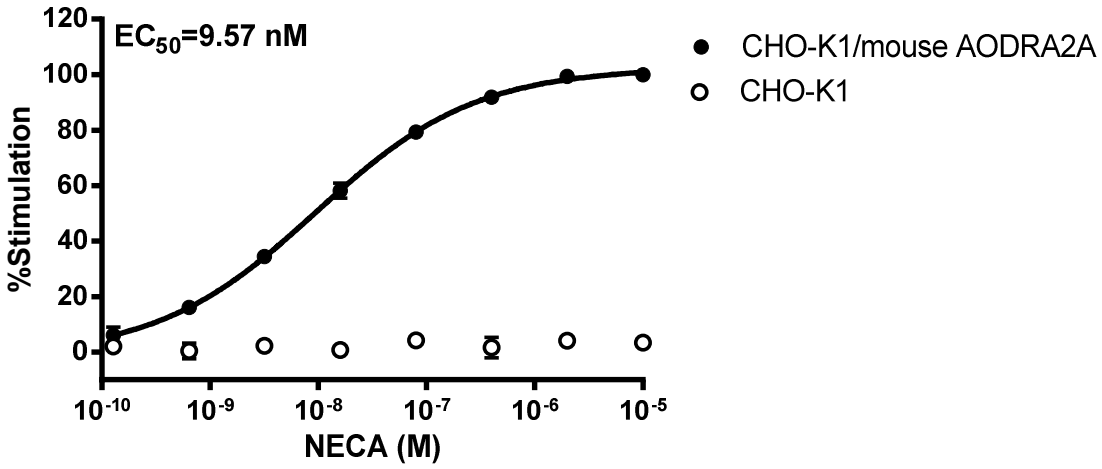

Figure 1. Dose dependent stimulation of intracellular cAMP mobilization upon treatment with NECA. The EC_{50} of NECA on CHO-K1/mouse ADORA2A cells was 9.57 nM.

Agonist Assay Protocol

- Seed 5 µl of CHO-K1/mouse ADORA2A cells into a 384-well low volume plate, 4,000 cells per well, which in assay medium
- Add 5 µl of agonist to each well and incubate the plate for 30 min at 23°C.
- Add 5ul of cAMP-d2 lysis buffer solution to each well.
- Add 5µl of cAMP-AB lysis buffer solution to each well.
- Incubate the plate in the dark for one hour at 23°C.
- Read the plate PHERAstar PLUS (BMG Labtech, Offenburg, Germany).

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-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 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. 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 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. Olah ME. (1997) dentification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of chimeric A1/A2a adenosine receptors. *J Biol Chem.* 272(1):337-44.
2. Bosch MP *et al.* (2004) Synthesis and biological activity of new potential agonists for the human adenosine A2A receptor. *J Med Chem.* 47(16):4041-53.

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