Human Recombinant Adenosine A2B Receptor Stable Cell Line
Cat. No. M00329 **Version 07292020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for ADORA2B receptor (Calcium flux assay, cAMP assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 µg/ml G418 (Cat. #10131-035, Gibco), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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 dependents 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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Calcium mobilization assay:

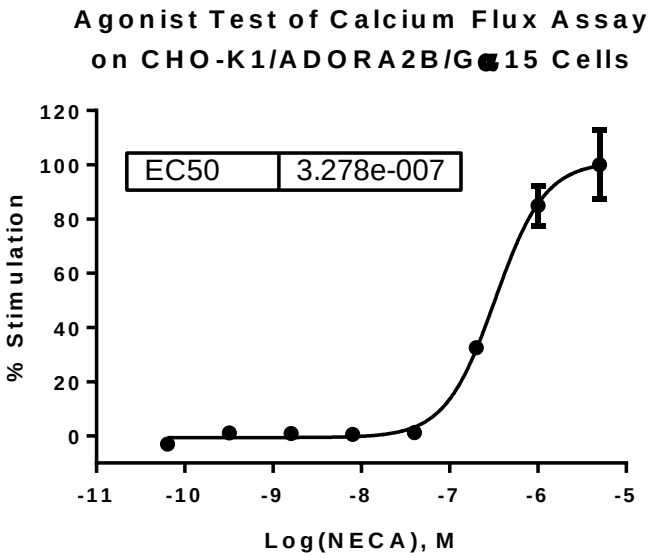

Figure 1. NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA2B/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist NECA. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of NECA (Mean ± SD, n = 2). The EC_{50} of NECA on this cell was 0.33 µM.

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.

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.

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
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.
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.
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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