Human Recombinant Adenosine A2A Receptor Stable Cell Line

Technical Manual No. TM0535

I. Introduction

Catalog Number: M00246
Cell Line Name: CHO-K1/ADORA2A/Gα15
Gene Synonyms: ADORA2A; ADORA2; RDC8; hA2AR
Expressed Gene: Genbank Accession Number NM_000675; 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 ADORA2A 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, 100 μg/ml Hygromycin B
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. Background

The adenosine receptors ADORA2A is 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.
III. Representative Data
Concentration-dependent stimulation of intracellular calcium mobilization by NECA in CHO-K1/ADORA2A/Gα15 and CHO-K1/Gα15 cells

Figure 1. NECA-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/ADORA2A/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an ADORA2A 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 ADORA2A co-expressing with Gα15 in CHO-K1 cells was 8.8 nM. The S/B of NECA on ADORA2A co-expressing with Gα15 in CHO-K1 cells was 4.

Notes:
1. EC_{50} value is calculated with four parameter logistic equation:
   \[ Y = \text{Bottom} + \left( \text{Top} - \text{Bottom} \right) / \left( 1 + 10^{((\log EC_{50} - X) \times \text{HillSlope})} \right) \]
   \( X \) 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and Zeocin to concentrations of 100 μg/ml and 200 μg/ml respectively the following day.
Subculturing: Protocol

1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References

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