

Human Recombinant Adenosine receptor A1 Stable Cell Line**Cat. No. M00255****Version 05282014**

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I. INTRODUCTION

Catalog Number: M00255

Cell Line Name: 293/ADORA1/ Gα15

Gene Synonyms: ADORA1

Expressed Gene: Genbank Accession Number NM_000674; no expressed tags

Host cell: 293

Quantity: 2 vials (3×10⁶ per vial) frozen cells

Stability: 16 passages

Application: Functional assay for A1 receptor

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete growth medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 µg/mL Zeocin, 100µg/mL Hygromycin-B

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.

860 Centennial Ave., Piscataway, NJ 08854, USA

Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com
§: 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. pultrhonis*, *M. arthritis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma (U. urealyticum)*, with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

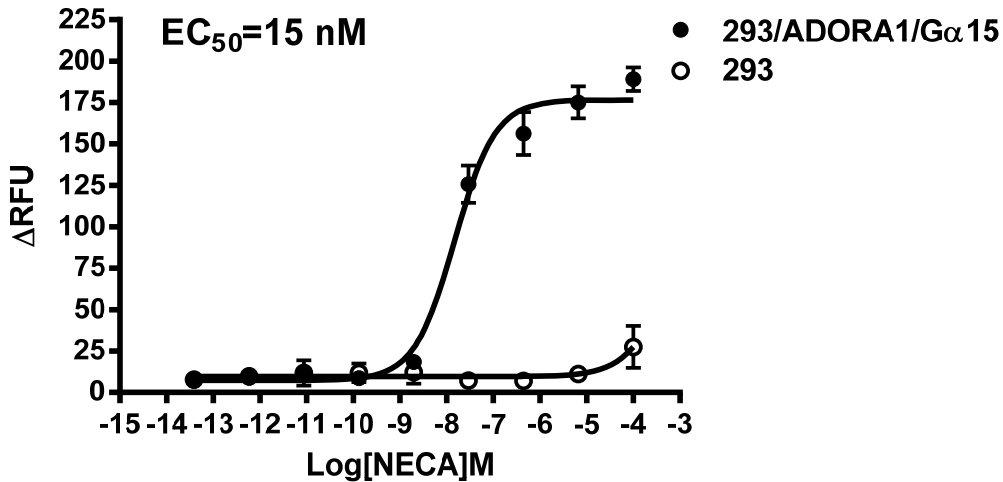


Figure. Intracellular calcium response from 293 cells stably co-expressing human recombinant adenosine receptor A1 and Gα15 and from untransfected control cells. Cells were loaded with Calcium-4 then stimulated with the indicated concentrations of NECA. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations. The EC₅₀ of NECA on ADORA1 co-expressing with Gα15 in CHO-K1 cells was 15 nM. The S/B of NECA on ADORA1 co-expressing with Gα15 in CHO-K1 was 24.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope})}}$$
 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.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO₂.
- 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. 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.

GenScript USA Inc,

860 Centennial Ave.

Piscataway, NJ 08854

Toll-Free: 1-877-436-7274

Tel: 1-732-885-9188, Fax: 1-732-210-0262

Email: product@genscript.com

Web: <http://www.genscript.com>

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