Human Recombinant P2Y2 Purinergic Receptor Stable Cell Line

Cat. No. M00318 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00318
Cell Line Name: 1321N1/P2Y2
Gene Synonyms: HP2U; MGC20088; MGC40010; P2RU1; P2U; P2U1; P2UR; P2Y2; P2Y2R
Expressed Gene: Genbank Accession Number NM_176071; no expressed tags
Host Cell: 1321N1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: Functional assay for P2Y2 receptor
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Complete Growth Medium: DMEM, 10% FBS
Culture Medium: DMEM, 10% FBS, 250 µg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery.

II. BACKGROUND

P2Y receptors are members of a large family of G protein-coupled receptors that is classified into five functionally characterized human gene products (P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11).
The P2Y2 receptor, originally called P2U purinoceptor, recognizes both ATP and UTP as the most potent agonists. It ranges from excitation of sympathetic neurons, muscle cell proliferation, endothelial cell adhesion, and spermiogenesis to acid-base equilibrium in intestinal epithelial cells and mucociliary clearance in normal and cystic fibrosis airway epithelia. In the airways, the P2Y2 and the P2Y6 receptors on the apical surface of airway epithelial cells control several of the calcium and protein kinase C dependent components of mucociliary clearance. The P2Y2 receptor is also expressed on the basolateral cell surface along with the P2Y1 receptor.

§: 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 ATP in 1321N1/P2Y2 and 1321N1 cells

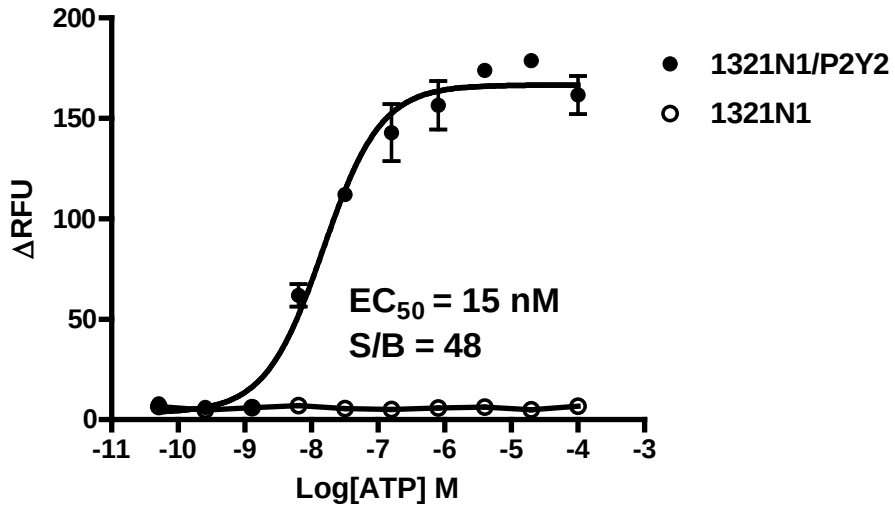

Figure 1. ATP-induced concentration-dependent stimulation of intracellular calcium mobilization in 1321N1/P2Y2 and 1321N1 cells. The cells were loaded with Calcium-4 prior to stimulation with a P2Y2 receptor agonist, 1321N1. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of ATP (Mean ± SD, n = 2). The EC_{50} of ATP on P2Y2 in CHO-K1 cells was 15 nM. he S/B of ATP on P2Y2 in 1321N1 cells was 48.

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. Add antibiotic in the following day.

Sub-culturing 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. Anderson CM and Parkinson FE. (1997) Potential signaling roles for UTP and UDP: sources, regulation and release of uracil nucleotides. *Trends Pharmacol Sci* 18: 387–392.
2. Boarder MR and Hourani SM. (1998) The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Trends Pharmacol Sci* 19: 99–107.
3. Communi D and Boeynaems JM. (1997) Receptors responsive to extracellular pyrimidine nucleotides. *Trends Pharmacol Sci* 18: 83–86.

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