Human Recombinant P2Y2 Purinergic Receptor Stable Cell Line

Technical Manual No. TM0509 **Version 10132010**

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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.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by ATP in 1321N1/P2Y2 and 1321N1 cells

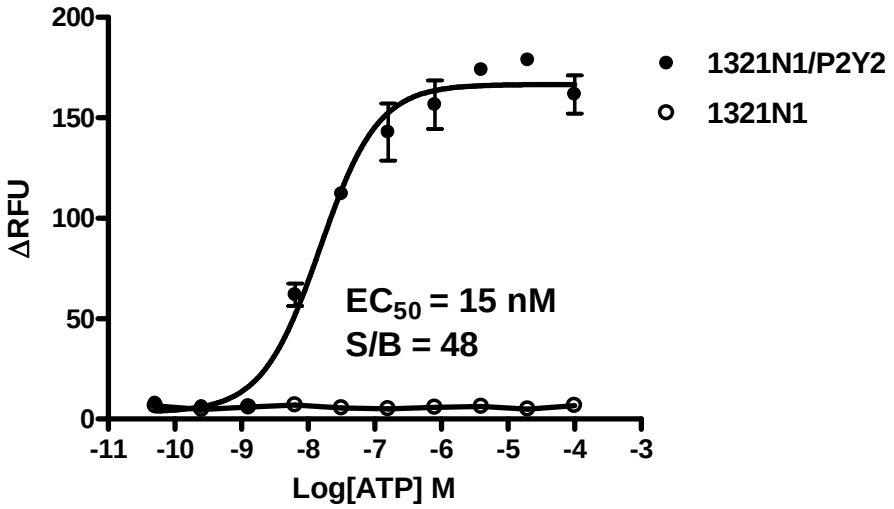

Figure 1. ATP-induced concentration-dependent stimulation of intracellular calcium mobilization in 1321N1/P2Y2 and 1321N1 cells. The cells were loaded with Calcium-4 prior to stimulation with a P2Y2 receptor agonist, 1321N1. The intracellular calcium change was measured by FlexStation. The relative fluorescent 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. The 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 the 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 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 G418 to a concentration of 250 µg/ml the following day.

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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 a 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 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

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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