
Human Recombinant P2Y11 Purinergic Receptor Stable Cell Line

Technical Manual No. TM0441

Version 10132010

I	Introduction	1
II	Background.....	1
III	Representative Data.....	2
IV	Thawing and Subculturing.....	2
V	References	3
	Limited Use License Agreement.....	4

I. Introduction

Catalog Number: M00298

Cell Line Name: P2Y11

Gene Synonyms: P2RY11

Expressed Gene: Genbank Accession Number NM_002566; no expressed tags

Host Cell: 1321N1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for P2Y11 receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 250 μ g/mL G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

P2Y11 is a receptor binding to ATP and ADP. It mediates both mobilization of intracellular calcium and stimulation of adenylate cyclase. The P2Y11-receptor may play a role in the differentiation of immunocytes and maturation of human monocyte-derived dendritic cells.

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by ATP in 1321N1/P2Y11 and 1321N1 cells

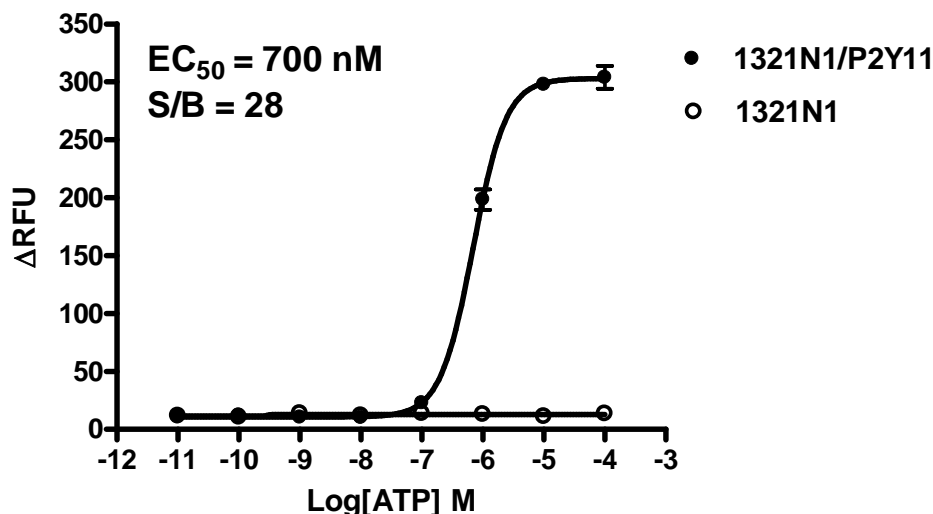


Figure 1. ATP-induced concentration-dependent stimulation of intracellular calcium mobilization in 1321N1/P2Y11 and 1321N1 cells. The cells were loaded with Calcium-4 prior to stimulation with a P2Y11 receptor agonist, ATP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of ATP (Mean \pm SD, n = 2). The EC₅₀ of ATP on P2Y11 in 1321N1 cells was 700 nM. The S/B of ATP on P2Y11 in 1321N1 cells was 28.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope})})$$

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 discard the medium.
- Resuspend the cells in complete growth medium.
- Add 10 ml of the cell suspension in a 10 cm dish.
- Add G418 to a concentration of 250 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

1. White PJ, *et al.* (2003) Characterization of a Ca²⁺ response to both UTP and ATP at human P2Y₁₁ receptors: evidence for agonist-specific signaling. *Mol. Pharmacol.* 63(6):1356-63.
2. van der Weyden L, *et al.* (2000) Signal transduction and white cell maturation via extracellular ATP and the P2Y₁₁ receptor. *Immunol. Cell Biol.* 78(4):369-74.
3. von Kügelgen I, *et al.* (2000) Molecular pharmacology of P2Y-receptors. *Naunyn. Schmiedeberg's. Arch. Pharmacol.* 362(4-5):310-23.

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