

Human Recombinant P2Y1 Purinergic Receptor Stable Cell Line

Technical Manual No. TM0598

Version 10132010

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I. Introduction

Catalog Number: M00250

Cell Line Name: 1321N1/P2Y1

Gene Synonyms: P2RY1; P2Y1

Expressed Gene: Genbank Accession Number NM_002563; no expressed tags

Host Cell: 1321N1

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

Stability: 16 passages

Application: Functional assay for P2Y1 receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 200 μ g/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

Purinergic receptor P2Y1 belongs to the family of G-protein coupled receptors. This family has several receptor subtypes with different pharmacological selectivity, which overlaps in some cases, for various adenosine and uridine nucleotides. This receptor functions as a receptor for extracellular ATP and ADP. In platelets binding to ADP leads to mobilization of intracellular calcium ions via activation of phospholipase C, a change in platelet shape, and probably to platelet aggregation.

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by ADP in 1321N1/P2Y1 and 1321N1 cells

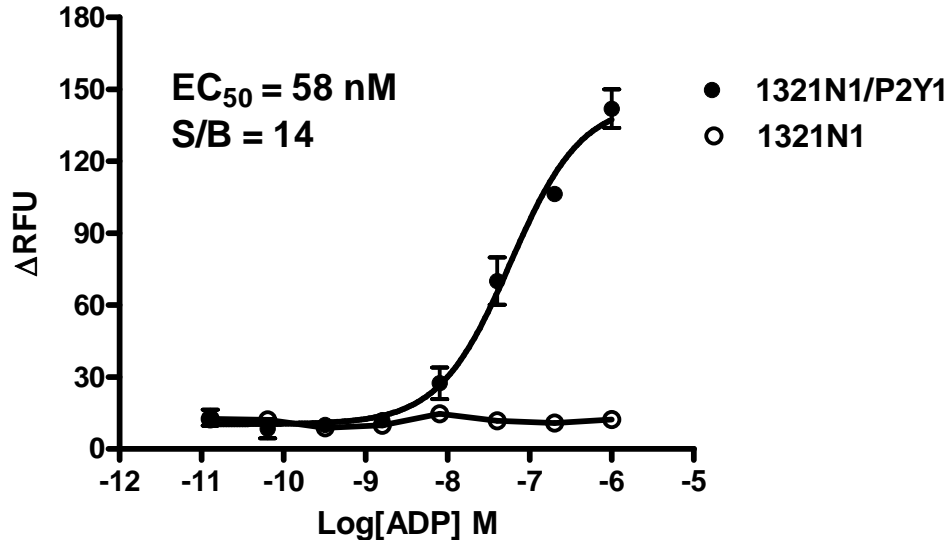


Figure 1. ADP-induced concentration-dependent stimulation of intracellular calcium mobilization in 1321N1/P2Y1 and 1321N1 cells. The cells were loaded with Calcium-4 prior to stimulation with a P2Y1 receptor agonist, ADP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of ADP (Mean \pm SD, n = 2). The EC₅₀ of ADP on P2Y1 in 1321N1 cells was 58 nM. The S/B of ADP on P2Y1 in 1321N1 cells was 14.

Notes:

1. EC₅₀ value is calculated with four parameter logistic equation:

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

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.

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 Zeocin to a concentration of 200 µg/ml 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. Nisar S, *et al.* (2010) Regulation of P2Y1 receptor traffic by sorting Nexin 1 is retromer independent. *Traffic*. 11(4):508-19.
2. Bambace NM, *et al.* (2010) The effect of P2Y-mediated platelet activation on the release of VEGF and endostatin from platelets. *Platelets*. 21(2):85-93.

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