Human Recombinant P2Y12 Purinergic Receptor Stable Cell Line

Technical Manual No. TM0578 **Version 07072010**

I. Introduction

Catalog Number: M00261

Cell Line Name: 1321N1/P2Y12/Gα15

Gene Synonyms: P2RY12; ADPG-R; HORK3; P2Y12; SP1999

Expressed Gene: Genbank Accession Number NM_022788; no expressed tags

Host Cell: 1321N1

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

Stability: 16 passages

Application: Functional assay for P2Y12 receptor

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

Complete Growth Medium: DMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 50 μg/ml Hygromycin B, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The P2Y receptor 12 (P2Y12) is a G_i-coupled GPCR expressed in platelets, brain tissues, vascular smooth muscle cells. P2Y12 can sustain platelet aggregation and promote thrombus growth and stabilization. It also plays a function in dense and alpha granule secretion, p-selectin expression and microparticle formation.

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III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by ADP in 1321N1/P2Y12/Gα15 and 1321N1 cells

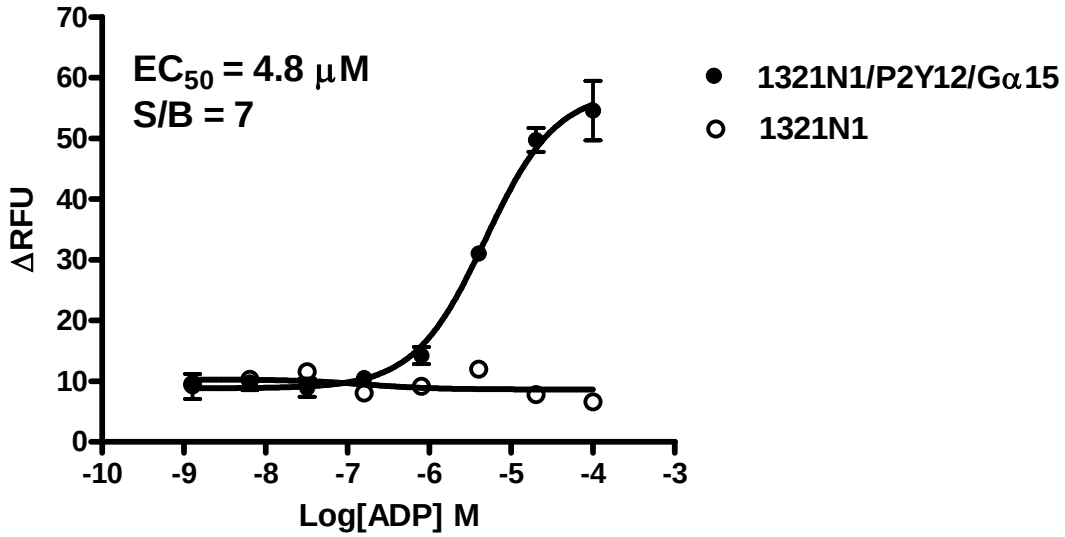

Figure 1. ADP-induced concentration-dependent stimulation of intracellular calcium mobilization in 1321N1/P2Y12/Gα15 and 1321N1 cells. The cells were loaded with Calcium-4 prior to stimulation with a P2Y12 receptor agonist, ADP. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of ADP (Mean ± SD, n = 2). The EC_{50} of ADP on P2Y12 in CHO-K1 cells was 4.8 μM. The S/B of ADP on P2Y12 in CHO-K1 cells was 7.

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 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 Hygromycin B and Zeocin to concentrations of 50 μg/ml and 200 μg/ml respectively 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 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. Hollopeter G, (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature.* 409(6817):202-7.

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