Human Recombinant P2Y12 Purinergic Receptor Stable Cell Line

Cat. No.: M00261 **Version 07012014**

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_0022788; 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, 200 μg/ml Zeocin, 50 μg/ml Hygromycin B
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

The P2Y receptor 12 (P2Y12) is a Gi-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 formationGenScript's human P2Y12-expressing stable cell is guaranteed to function properly in calcium flux assay.

§: 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

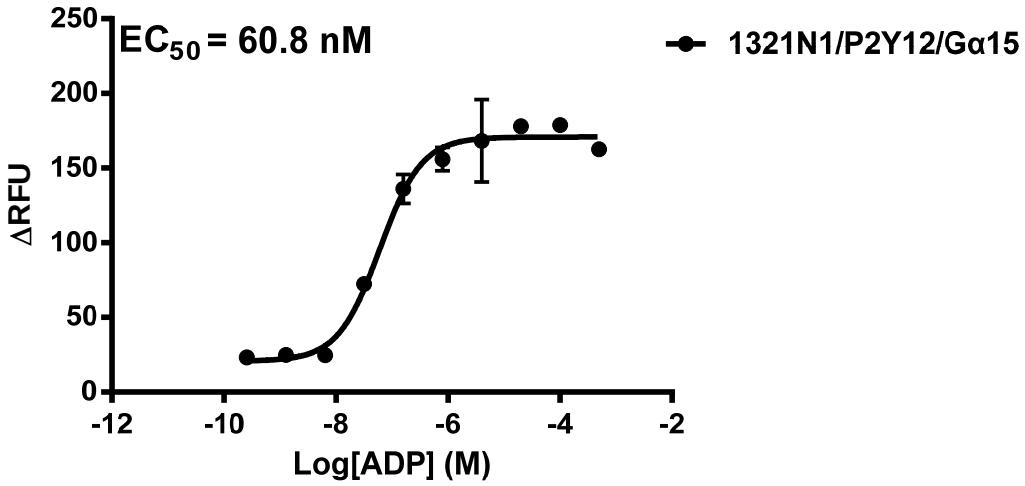

Figure Dose dependent stimulation of intracellular calcium mobilization upon treatment with its ligand ADP. The P2Y12-expressing stable subline (GenScript, Cat No: M00261) was loaded with Calcium-4 prior to stimulation with a P2Y12 receptor agonist, ADP. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses of ADP (Mean ± SD, n = 2). The EC_{50} of ADP on P2Y1 in 1321N1 cells was 60.8 nM.

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 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. In the following day, replace the cells with fresh medium contains antibiotic.

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. Hollopeter G, (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature.* 409(6817):202-7.

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