

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT P2Y₁ PURINERGIC RECEPTOR**

CATALOG NUMBER: HTS049C **QUANTITY:** 2 vials, 1 mL per vial

LOT NUMBER: **CONCENTRATION:** 2 x 10⁶ cells/mL

BACKGROUND: P2Y₁ is a GPCR that binds to ADP and ATP to activate G_q. A wide variety of cells and tissues express P2Y₁. Vascular endothelium and smooth muscle express P2Y₁, and mediate vascular tone both in the resting state and in thrombosis, when platelet-derived ATP and ADP are present at high concentrations. In addition, activation of P2Y₁ expressed on platelets leads to ADP-induced shape changes and aggregation (Ralevic and Burnstock, 1998). Chemicon's cloned human P2Y₁-expressing cell line is made in the 1321N1 host, which supports high levels of recombinant P2Y₁ expression on the cell surface. Thus, the cell line is an ideal tool for screening for antagonists of interactions between P2Y₁ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

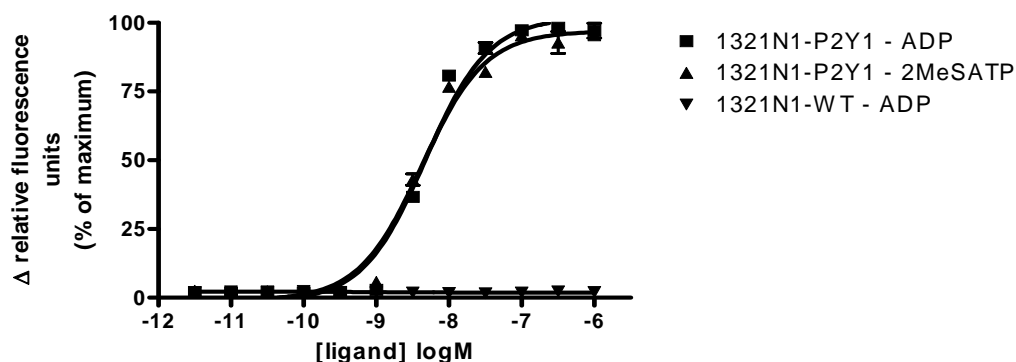


Figure 1. Calcium flux in P2Y₁-expressing 1321N1 cell line induced by ADP. P2Y₁-expressing 1321N1 cells were loaded with Fluo-4 NW dye (Invitrogen) and calcium flux in response to ADP (10⁻⁴ to 10⁻⁹ M) was determined in triplicate on a Molecular Devices Flex Station.

SPECIFICATIONS: EC50 for calcium mobilization by ADP: ~ 4.7 nM
EC50 for calcium mobilization by 2MeSATP: ~ 4.2nM

HOST CELLS: 1321N1, an adherent cell line lacking endogenous expression of ADP/ATP receptors.

Note: This P2Y₁ cell line in the 1321N1 background replaces the P2Y₁-Chem-3 cell line previously offered under this catalog number

TRANSFECTION: Full-length human P2Y₁ cDNA (Accession Number: NM_002563)

PRESENTATION: Cells are frozen at 2 x 10⁶ cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

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1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca⁺⁺ and Mg⁺⁺ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO₂ until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10⁶ cells/mL in 1321N1 Freezing Media (cell densities of 2-10 x 10⁶ are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in 1321N1 Plating Media for plating for calcium assay.

MEDIA:

1321N1 Growth Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250µg/mL Genetecin/G-418

1321N1 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

1321N1 Freezing Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

Ralevic V and Burnstock G (1998) Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50: 413-492.

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HUMAN RECOMBINANT P2Y₁ PURINERGIC RECEPTOR**

Product No. HTS049C

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