ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT $P2Y_2$ PURINERGIC RECEPTOR

CATALOG NUMBER:	HTS210C	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	RI08040034	**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

The P2Y GPCRs serve as receptors for extracellular nucleotides (Abbracchio *et al.,* 2006). $P2Y_2$ (also known as P_{2U}) is activated by UTP and ATP, and couples to G_q to increase intracellular calcium (Parr *et al*., 1994). The $P2Y_2$ receptor knockout mouse displays defective nucleotide-stimulated Cl^- secretion, and a $P2Y_2$ selective agonist increases tracheal epithelial chloride and water secretion (Cressman *et al*., 1999; Yerxa *et al*., 2002). As a result, $P2Y_2$ is a potential target for treatment of cystic fibrosis. In addition, the $P2Y_2$ KO mouse displays salt-resistant hypertension (Rieg *et al*., 2007). Millipore's cloned human $P2Y_2$-expressing cell line is made in the 1321N1host, which supports high levels of recombinant $P2Y_2$ expression on the cell surface. Thus, the cell line is an ideal tool for screening for agonists and antagonists of $P2Y_2$.

APPLICATIONS:

Calcium flux assay

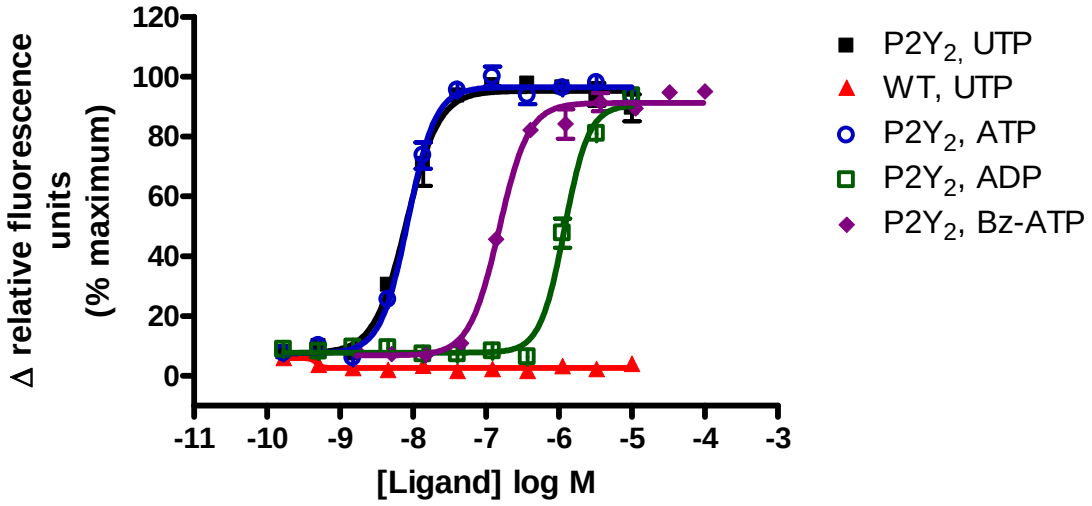

Figure 1. Calcium flux in $P2Y_2$–expressing 1321N1 cell line induced by UTP, ATP, ADP, and Benzoylbenzoyl-ATP (Bz-ATP). $P2Y_2$- expressing 1321N1 cells and Wild-Type 1321N1 cells were loaded with Fluo-8 NW Calcium Assay Kit and calcium flux in response to various ligands was determined in triplicate on a Molecular Devices FLIPR$^{TETRA_{TM}}$. In this experiment, average maximum signal was 3730 RLU. The Z' was 0.75 with UTP at 2x EC50.

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Table I. Comparison of EC50 values of $P2Y_2$-expressing 1321N1 cells with values described in the literature.

ligand	assay	potency (nM)	Reference
UTP	Calcium	EC50 = 8.2	Figure 1
UTP	Inositol phosphate	EC50 = 140	Lazarowski *et al*., 1995
ATP	Calcium	EC50 = 8.5	Figure 1
ATP	Inositol phosphate	EC50 = 230	Lazarowski *et al*., 1995
Bz-ATP	Calcium	EC50 = 152	Figure 1
ADP	Calcium	EC50 = 1193	Figure 1
ADP	Inositol phosphate	EC50 = ~2000	Nicholas *et al*., 1996

HOST CELLS: 1321N1, an adherent cell line lacking the endogenous expression of receptors for UTP.

TRANSFECTION: Proprietary plasmid containing full-length human P2RY2 cDNA encoding $P2Y_2$ (Accession Number: NM_002564.2). The stable clonal cell line was selected by resistance to geneticin, followed by limited dilution cloning. The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture.

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% FBS/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC 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 37ºC with 5% CO_2.
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 37ºC with 5% CO_2 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 1321N1 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 10^6 cells/mL in 1321N1 Freezing Media (cell densities of 2-10 x 10^6 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:
90% heat-inactivated FBS
10% DMSO (cell culture grade)

EXAMPLE ASSAY CONDITIONS:

1. Cells propagated for screening should be maintained and seeded at less than 90% confluency. Trypsinize cells as above and seed cells in 96-well black-walled, clear bottom plate at 50,000 cells/well in 1321N1 Plating Media. Keep the plate at room temperature for 1 h to allow even cell distribution in the plate, then transfer plate to a humidified incubator at 37ºC with 5% CO_2.
2. 1321N1 derived cell lines have been successfully assayed using multiple commercially-available calcium dye kits following the manufacture's protocols. The protocol described below is a suggested protocol that can be generally applied to most calcium dyes kits.
3. Remove media
4. Wash cells with buffered salt solution
5. Add 100 μL/well calcium dye-loading solution.

6. Incubate the plate for 30 minutes in a humidified incubator at 37ºC with 5% CO_2.
7. Incubate the plate for an additional 60 min at 25ºC with 5% CO_2.
8. Set-up FLIPR to dispense 50uL/well 3X ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height at 95 uL and dispense rate to 25 μL/sec. Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
9. Ligands are prepared in a white nonbinding surface 96-well plate (Corning 3605).
10. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

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