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PRODUCT DATASHEET

ChemiScreen™ $P2Y_4$ Purinergic Receptor Stable Cell Line

CATALOG NUMBER: HTS211C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the 1321N1 host, which supports high levels of functional receptor expression on the cell surface. 1321N1 cells contain high endogenous levels of G_q, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The P2Y GPCRs serve as receptors for extracellular nucleotides. The $P2Y_4$ receptor is activated by UTP, and also by ATP in rodents, and couples to G_q to increase intracellular calcium (von Kügelgen, 2006). $P2Y_4$ is expressed prominently in the intestinal epithelium, where it regulates intestinal chloride secretion. $P2Y_4$ therefore is likely to be involved in infectious diarrhea caused by nucleotides released upon infection with enteropathogenic bacteria (Robaye *et al*., 2003). The cloned human $P2Y_4$-expressing cell line is made in the 1321N1 host, which supports high levels of recombinant $P2Y_4$ expression on the cell surface. Thus, the cell line is an ideal tool for screening for agonists and antagonists of $P2Y_4$.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

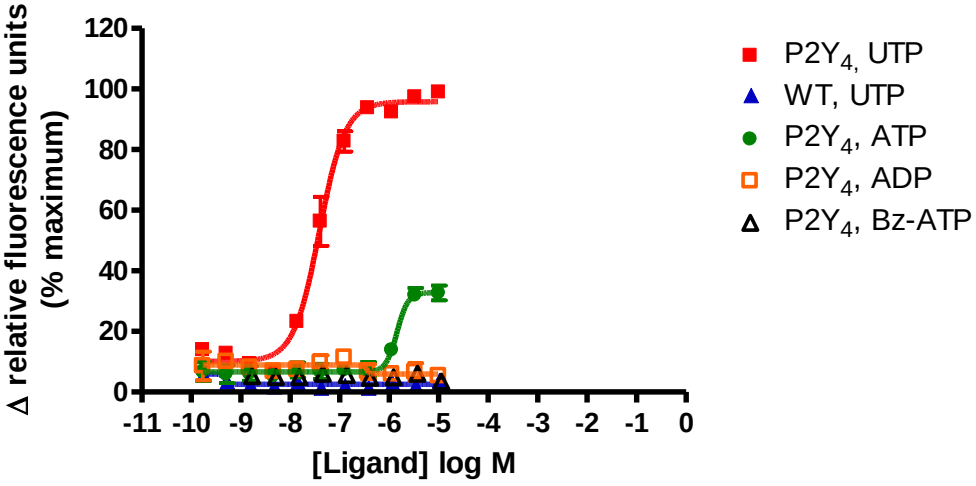

Figure 1. Representative data for activation of the $P2Y_4$ receptor stably expressed in 1321N1 cells induced by UTP, ATP, ADP, and Benzoylbenzoyl-ATP (Bz-ATP) using a fluorescent calcium flux assay. $P2Y_4$–expressing 1321N1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera.

Table 1. EC_{50} values of $P2Y_4$-expressing 1321N1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
UTP	Calcium Flux - Fluorescence	73.2	Eurofins Internal Data
ATP	Calcium Flux - Fluorescence	2500	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	10mM	Millipore Sigma: H3537
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Gibco:10131-027
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01

CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
UTP	Sigma: U6625
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

HOST CELL

1321N1, an adherent cell line lacking the endogenous expression of receptors for P2Y4.

EXOGENOUS GENE EXPRESSION

Full-length human P2RY4 cDNA encoding $P2Y_4$ (Accession Number: NM_002565.3) and promiscuous G_q protein are expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTS213C	ChemiScreen™ $P2Y_{11}$ Purinergic Receptor Stable Cell Line

REFERENCES

1. Communi D et al. (1995) Cloning and functional expression of a human uridine nucleotide receptor. J. Biol. Chem. 270: 30849-30852.
2. Communi D et al. (1996) Pharmacological characterization of the human P2Y4 receptor. Eur. J. Pharmacol. 317: 383-389.
3. Herold CL et al. (2004) Agonist versus antagonist action of ATP at the P2Y4 receptor is determined by the second extracellular loop. J. Biol. Chem. 279: 11456-11464.
4. Robaye B et al. (2003) Loss of nucleotide regulation of epithelium chloride transport in the jejunum of P2Y4-null mice. Mol. Pharmacol. 63:777-783.
5. von Kügelgen I (2006) Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. *Pharmacol. Ther.* 110: 415-432.

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