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# PRODUCT DATASHEET

## Ready-to-Assay™ $P2Y_4$ Purinergic Receptor Frozen Cells

## CATALOG NUMBER: HTS211RTA

**CONTENTS**: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

**STORAGE**: Vials are to be stored in liquid $N_2$. Media Component at 4ºC (-20ºC for prolonged storage).

## BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

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 Gq 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). 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 User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

## 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 Assays

## APPLICATION DATA

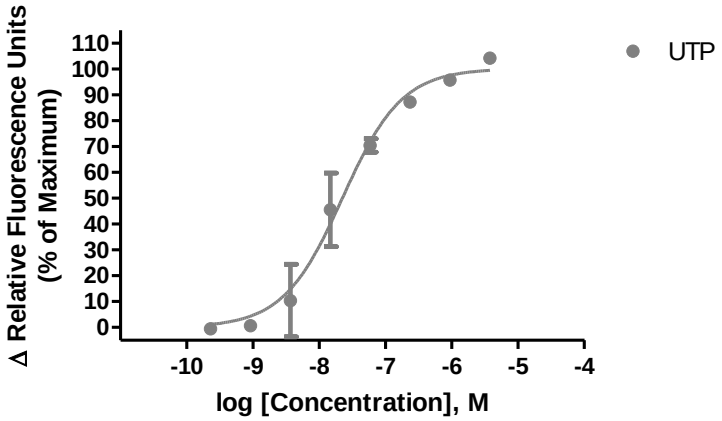


Figure 1. Representative data for activation of $P2Y_4$ receptor. Calcium flux in $P2Y_4$–expressing 1321N1 cell line induced by UTP. $P2Y_4$–expressing 1321N1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR$^{TETRA}$. Maximal fluorescence signal obtained in this experiment was 1,700 RLU (Relative Light Units).

Table 1. $EC_{50}$ value of P2Y4-expressing 1321N1 cells.

| LIGAND | ASSAY | POTENCY (nM) | REFERENCE |
|---|---|---|---|
| **UTP** | Calcium Flux | 22 | Eurofins Internal Data |

## ASSAY SETUP

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.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) $Ca^{2+}$ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW $Ca^{2+}$ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate ($Ca^{2+}$ dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 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 to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

## ASSAY MATERIALS

| Description | Supplier and Product Number |
|---|---|
| HBSS | Hyclone: SH30268.02 |
| HEPES 1M Stock | EMD Millipore.: TMS-003-C |
| Probenicid | Sigma: P8761 |
| Quest Fluo-8™, AM | AAT Bioquest: 21080 |
| UTP ligand | Sigma: U6625 |
| Non-binding white plates (for ligand prep) | Corning: 3605(96-well)/3574(384-well) |
| Black (clear bottom) tissue-culture treated plates | Corning: 3904(96-well)/3712(384-well) |

## FLIPR SETTINGS

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 | 25 µl (50 µl for 384-well) |
| Dispense Speed | 75 µl L/sec (50 µl for 384-well) |
| Expel Volume | 0 µl |
| Analysis | Subtract Bias Sample 1 |

# HOST CELL

1321N1, an adherent human astrocytoma cell line.

# EXONGENOUS GENE EXPRESSION

P2Y4 cDNA (Accession Number: NM_002565.3; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

```
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 S  E  V  E  L  D  C  W  F  D  E  D  F  K  F  I  L  L  P  V
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```

## RELATED PRODUCTS

| PRODUCT NUMBER | DESCRIPTION |
|---|---|
| **HTS1321N1RTA** | Ready-to-Assay™ 1321N1 host frozen cells (control cells) |

## 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 $P_{2Y4}$ receptor. *Eur. J. Pharmacol.* 317: 383-389.
3. Herold CL *et al.* (2004) Agonist versus antagonist action of ATP at the $P2Y_4$ 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 $P2Y_4$-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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