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# PRODUCT DATASHEET

## Ready-to-Assay™ $P2Y_1$ Purinergic Receptor Frozen Cells

## CATALOG NUMBER: HTS049RTA

**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.

$P2Y_1$ is a GPCR that binds to ADP and ATP to activate Gq. A wide variety of cells and tissues express $P2Y_1$. Vascular endothelium and smooth muscle express $P2Y_1$, 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 $P2Y_1$ expressed on platelets leads to ADP-induced shape changes and aggregation (Ralevic and Burnstock, 1998). Cloned human $P2Y_1$-expressing cell line is made in the 1321N1 host, which supports high levels of recombinant $P2Y_1$ expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at $P2Y_1$.

## 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

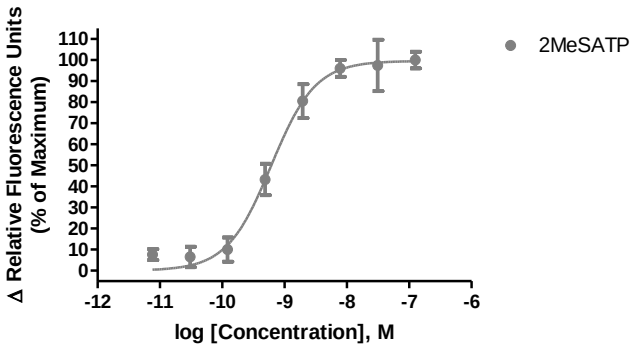


Figure 1. Representative data for activation of $P2Y_1$ receptor. Calcium flux in $P2Y_1$–expressing 1321N1 cell line induced by 2MeSATP. $P2Y_1$–expressing 1321N1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR$^{TETRA}$ with ICCD camera. Maximal fluorescence signal obtained in this experiment was 25,000 RLU (Relative Light Units).

Table 1. $EC_{50}$ value of $P2Y_1$-expressing 1321N1 cells.

| LIGAND | ASSAY | POTENCY (nM) | REFERENCE |
|---|---|---|---|
| **2MeSATP** | Calcium Flux | 0.6 | 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 |
| 2MeSATP ligand | Tocris: 1062 |
| Non-binding white plates (for ligand prep) | Corning: 3605(96-well)/3574(384-well) |
| Collagen black (clear bottom) tissue-culture treated plates | BD Falcon: 356649 |

## 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

P2RY1 cDNA (Accession Number: NM_002563; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

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ATG ACC GAG GTG CTG TGG CCG GCT GTC CCC AAC GGG ACG GAC GCT GCC TTC CTG GCC GGT CCG GGT TCG TCC TGG GGG AAC AGC
ACG GTC GCC TCC ACT GCC GCC GTC TCC TCG TCG TTC AAA TGC GCC TTG ACC AAG ACG GGC TTC CAG TTT TAC TAC CTG CCG GCT
GTC TAC ATC TTG GTA TTC ATC ATC GGC TTC CTG GGC AAC AGC GTG GCC ATC TGG ATG TTC GTC TTC CAC ATG AAG CCC TGG AGC
GGC ATC TCC GTG TAC ATG TTC AAT TTG GCT CTG GCC GAC TTC TTG TAC GTG CTG ACT CTG CCA GCC CTG ATC TTC TAC TAC TTC
AAT AAA ACA GAC TGG ATC TTC GGG GAT GCC ATG TGT AAA CTG CAG AGG TTC ATC TTT CAT GTG AAC CTC TAT GGC AGC ATC TTG
TTT CTG ACA TGC ATC AGT GCC CAC CGG TAC AGC GGT GTG GTG TAC CCC CTC AAG TCC CTG GGC CGG CTC AAA AAG AAG AAT GCG
ATC TGT ATC AGC GTG CTG GTG TGG CTC ATT GTG GTG GTG GCG ATC TCC CCC ATC CTC TTC TAC TCA GGT ACC GGG GTC CGC AAA
AAC AAA ACC ATC ACC TGT TAC GAC ACC ACC TCA GAC GAG TAC CTG CGA AGT TAT TTC ATC TAC AGC ATG TGC ACG ACC GTG GCC
ATG TTC TGT GTC CCC TTG GTG CTG ATT CTG GGC TGT TAC GGA TTA ATT GTG AGA GCT TTG ATT TAC AAA GAT CTG GAC AAC TCT
CCT CTG AGG AGA AAA TCG ATT TAC CTG GTA ATC ATT GTA CTG ACT GTT TTT GCT GTG TCT TAC ATC CCT TTC CAT GTG ATG AAA
ACG ATG AAC TTG AGG GCC CGG CTT GAT TTT CAG ACC CCA GCA ATG TGT GCT TTC AAT GAC AGG GTT TAT GCC ACG TAT CAG GTG
ACA AGA GGT CTA GCA AGT CTC AAC AGT TGT GTG GAC CCC ATT CTC TAT TTC TTG GCG GGA GAT ACT TTC AGA AGG AGA CTC TCC
CGA GCC ACA AGG AAA GCT TCT AGA AGA AGT GAG GCA AAT TTG CAA TCC AAG AGT GAA GAC ATG ACC CTC AAT ATT TTA CCT GAG
TTC AAG CAG AAT GGA GAT ACA AGC CTG TGA
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## RELATED PRODUCTS

| PRODUCT NUMBER | DESCRIPTION |
|---|---|
| **HTS131N1RTA** | Ready-to-Assay™ 1321N1 host frozen cells (control cells) |

## REFERENCES

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

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION



## User Agreement (Label License)

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