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PRODUCT DATASHEET

Ready-to-Assay™ $P2Y_{11}$ Purinergic Receptor Frozen Cells

CATALOG NUMBER: HTS213RTA

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 over night recovery, assays for calcium response.

The P2Y GPCRs serve as receptors for extracellular nucleotides (Abbracchio *et al.,* 2006). $P2Y_{11}$ is a relatively low affinity receptor for ATP present humans but not rodents. It is unique among P2Y receptors in increasing both cAMP and calcium levels (Qi *et al.,* 2001). $P2Y_{11}$ is expressed in immune cells, and it mediates dendritic cell maturation induced by ATP (Wilkin *et al*., 2001). In addition to ATP, NAD^+ and $NAADP^+$ bind and activate $P2Y_{11}$ in granulocytes (Moreschi *et al.,* 2006, 2007). An alternate form of $P2Y_{11}$, in which the second exon of $P2Y_{11}$ undergoes intergenic splicing with exon 12 of the adjacent SSF1 gene to generate a chimeric protein, is widely expressed in human tissues (Communi *et al*., 2001). Cloned human $P2Y_{11}$-expressing cell line is made in the 1321N1 host, which supports high levels of recombinant $P2Y_{11}$ expression on the cell surface. Thus, the cell line is an ideal tool for screening for agonists and antagonists of $P2Y_{11}$.

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

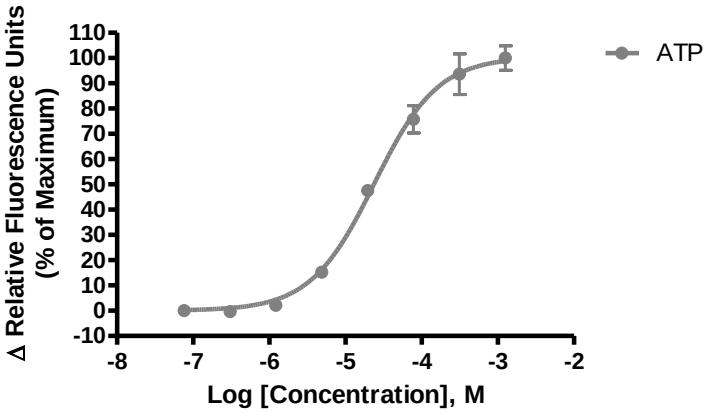

Figure 1. Representative data for activation of $P2Y_{11}$ receptor. Calcium flux in $P2Y_{11}$–expressing 1321N1 cell line induced by ATP. $P2Y_{11}$–expressing 1321N1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 8,000 RLU (Relative Light Units).

Table 1. EC_{50} value of $P2Y_{11}$-expressing 1321N1 cells.

LIGAND	ASSAY	POTENCY (µM)	REFERENCE
ATP	Calcium Flux	24	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
ATP ligand	Sigma: A6419
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Collagen black (clear bottom) tissue-culture treated plates	BD Falcon: 354649(96-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

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HOST CELL

1321N1, an adherent human astrocytoma cell line

EXONGENOUS GENE EXPRESSION

Human $P2Y_{11}$ cDNA (Accession Number: NM_002566; see CODING SEQUENCE below)

CODING SEQUENCE

```
ATG GCA GCC AAC GTC TCG GGT GCC AAG TCC TGC CCT GCC AAC TTC TTG GCA GCT GCC GAC GAC AAA CTC AGT
 M   A   A   N   V   S   G   A   K   S   C   P   A   N   F   L   A   A   A   D   D   K   L   S

GGG TTC CAG GGG GAC TTC CTG TGG CCC ATA CTG GTG GTT GAG TTC CTG GTG GCC GTG GCC AGC AAT GGC CTG
 G   F   Q   G   D   F   L   W   P   I   L   V   V   E   F   L   V   A   V   A   S   N   G   L

GCC CTG TAC CGC TTC AGC ATC CGG AAG CAG CGC CCA TGG CAC CCC GCC GTG GTC TTC TCT GTC CAG CTG GCA
 A   L   Y   R   F   S   I   R   K   Q   R   P   W   H   P   A   V   V   F   S   V   Q   L   A

GTC AGC GAC CTG CTC TGC GCC CTG ACG CTG CCC CCG CTG GCC GCC TAC CTC TAT CCC CCC AAG CAC TGG CGC
 V   S   D   L   L   C   A   L   T   L   P   P   L   A   A   Y   L   Y   P   P   K   H   W   R

TAT GGG GAG GCC GCG TGC CGC CTG GAG CGC TTC CTC TTC ACC TGC AAC CTG CTG GGC AGC GTC ATC TTC ATC
 Y   G   E   A   A   C   R   L   E   R   F   L   F   T   C   N   L   L   G   S   V   I   F   I

ACC TGC ATC AGC CTC AAC CGC TAC CTG GGC ATC GTG CAC CCC TTC TTC GCC CGA AGC CAC CTG CGA CCC AAG
 T   C   I   S   L   N   R   Y   L   G   I   V   H   P   F   F   A   R   S   H   L   R   P   K

CAC GCC TGG GCC GTG AGC GCT GCC GGC TGG GTC CTG GCC GCC CTG CTG GCC ATG CCC ACA CTC AGC TTC TCC
 H   A   W   A   V   S   A   A   G   W   V   L   A   A   L   L   A   M   P   T   L   S   F   S

CAC CTG AAG AGG CCG CAG CAG GGG GCG GGC AAC TGT AGC GTG GCC AGG CCC GAG GCC TGC ATC AAG TGT CTG
 H   L   K   R   P   Q   Q   G   A   G   N   C   S   V   A   R   P   E   A   C   I   K   C   L

GGG ACA GCA GAC CAC GGG CTG GCG GCC TAC AGA GCG TAT AGC CTG GTG CTG GCG GGG TTG GGC TGC GGC CTG
 G   T   A   D   H   G   L   A   A   Y   R   A   Y   S   L   V   L   A   G   L   G   C   G   L

CCG CTG CTG CTC ACG CTG GCA GCC TAC GGC GCC CTC GGG CGG GCC GTG CTA CGC AGC CCA GGC ATG ACT GTG
 P   L   L   L   T   L   A   A   Y   G   A   L   G   R   A   V   L   R   S   P   G   M   T   V

GCC GAG AAG CTG CGT GTG GCA GCG TTG GTG GCC AGT GGT GTG GCC CTC TAC GCC AGC TCC TAT GTG CCC TAC
 A   E   K   L   R   V   A   A   L   V   A   S   G   V   A   L   Y   A   S   S   Y   V   P   Y

CAC ATC ATG CGG GTG CTC AAC GTG GAT GCT CGG CGG CGC TGG AGC ACC CGC TGC CCG AGC TTT GCA GAC ATA
 H   I   M   R   V   L   N   V   D   A   R   R   R   W   S   T   R   C   P   S   F   A   D   I

GCC CAG GCC ACA GCA GCC CTG GAG CTG GGG CCC TAC GTG GGC TAC CAG GTG ATG CGG GGC CTC ATG CCC CTG
 A   Q   A   T   A   A   L   E   L   G   P   Y   V   G   Y   Q   V   M   R   G   L   M   P   L

GCC TTC TGT GTC CAC CCT CTA CTC TAC ATG GCC GCA GTG CCC AGC CTG GGC TGC TGC TGC CGA CAC TGC CCC
 A   F   C   V   H   P   L   L   Y   M   A   A   V   P   S   L   G   C   C   C   R   H   C   P

GGC TAC AGG GAC AGC TGG AAC CCA GAG GAC GCC AAG AGC ACT GGC CAA GCC CTG CCC CTC AAT GCC ACA GCC
 G   Y   R   D   S   W   N   P   E   D   A   K   S   T   G   Q   A   L   P   L   N   A   T   A

GCC CCT AAA CCG TCA GAG CCC CAG TCC CGT GAG CTG AGC CAA TGA
 A   P   K   P   S   E   P   Q   S   R   E   L   S   Q  Stp
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RELATED PRODUCTS

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

REFERENCES

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7. Wilkin F *et al.* (2001) The $P2Y_{11}$ receptor mediates the ATP-induced maturation of human moncyte-derived dendritic cells. *J. Immunol.* 166: 7172-7177.

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