GeneBLAzer® P2RY11 CHO-K1 DA Assay Kit

GeneBLAzer® P2RY11 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1353 and K1729

Cell Line Descriptions

GeneBLAzer® P2RY11 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells contain the human purinergic receptor P2, G protein-coupled, 11 (P2RY11) receptor (Accession # NM_002566) stably integrated into the CellSensor® NFAT-*bla* CHO-K1 cell line. CellSensor® NFAT-*bla* CHO-K1 cells (Cat. no. K1534) contain a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells (NFAT) response element. Division Arrested (DA) cells are available in as an Assay Kit (which includes cells and sufficient substrate to analyze 1 x 384-well plate.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both GeneBLAzer® P2RY11 CHO-K1 DA cells and GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of adenosine-5'-triphosphate (ATP); (Figure 1). In addition, GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also included.

Target Description

The P2Y receptor family is part of a larger receptor family whose physiological effects are mediated by extracellular nucleotide di- and tri-phosphates. The P2 receptor family consists of ion-gated channel receptors (P2X) and G protein-coupled receptors (P2Y). Currently there are five classified P2RY receptors (P2RY1, P2RY2, P2RY4, P2RY6, and P2RY11) with additional orphan receptors, P2RY5, P2RY9, and P2RY10 (1).

The P2RY11 receptor was originally cloned from human placenta and has 33% amino acid homology to P2RY1 and 28% amino acid homology to P2RY2 (2). P2RY11 expression is broadly distributed and has been found in the brain, pituitary, lymphocytes, spleen, intestines, macrophages, lung, stomach, adipose, pancreas, kidney, prostrate, heart, placenta, liver, skeletal muscle (3). Cell differentiation of human promyelocytic HL60 cells (4) and maturation of monocyte-derived dendritic cells have been shown to be mediated by ATP activation of P2RY11 (5).

The P2RY11 receptor signaling is coupled to both the phospholipase C and cAMP pathways (2, 6, 7). The primary endogenous agonist for P2RY11 is ATP with minimal activity in response to ADP, while uridine nucleotides were initially thought to be inactive. UTP has recently been shown to be linked to an increase in cytosolic Ca^{2+} concentration via a mechanism independent of the signaling pathways activated by ATP (9).

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. ATP agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1 µM	1.2 µM
Z'-factor	0.88	0.74

Recommended cell no. = 10K cells/well
DMSO Tolerance = up to 1.0%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 20 µM

2. Alternate agonist dose response

UTP EC_{50} = N/A
ADP EC_{50} = 7.6 µM
UDP EC_{50} = N/A

3. Antagonist dose response

Suramin IC_{50} = 7.8 µM

4. Agonist 2nd messenger dose response

ATP EC_{50} = 403 nM
UTP EC_{50} = 393 nM
ADP EC_{50} = 3.2 µM
UDP EC_{50} = 17 µM

Assay Testing Summary

5. Assay performance with variable cell number

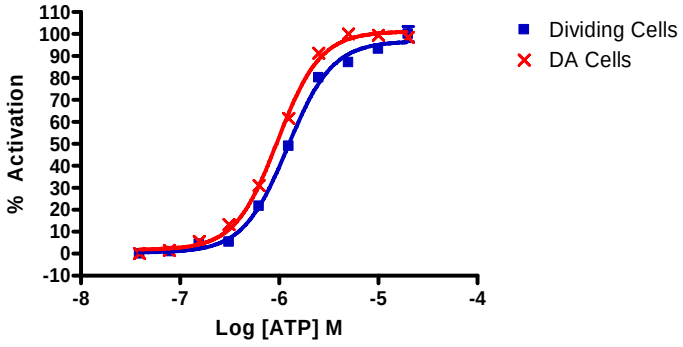
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

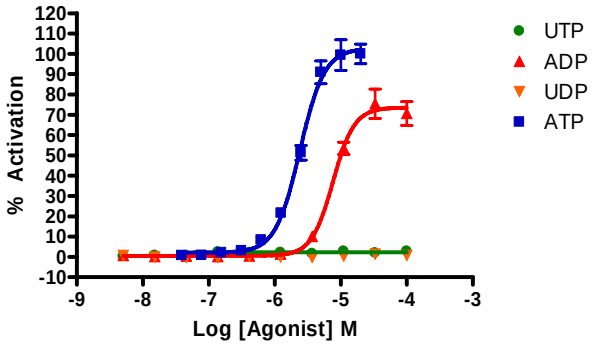
Figure 1 — GeneBLAzer® P2RY11 CHO-K1 DA and GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP under optimized conditions



GeneBLAzer® P2RY11 CHO-K1 DA cells and GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of ATP in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and % Activation plotted for each replicate against the concentrations of ATP(n=6 for each data point).

Alternate Agonist Dose Response

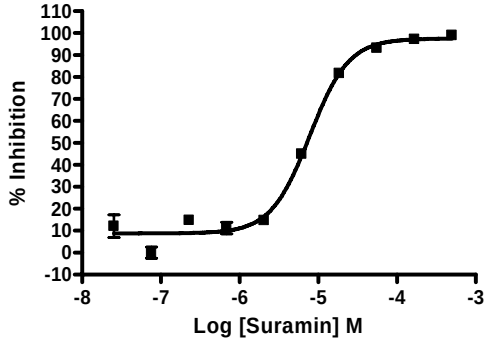
Figure 2 — GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP, UTP, ADP and UDP under optimized conditions



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells were plated at 10,000 cells/well in a 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of adenosine-5'-triphosphate (Sigma cat# A7699), uridine-5'-triphosphate (Sigma #U1006), adenosine-diphosphate (Sigma #A2754), and uridine-diphosphate (Sigma #U4125) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained and the % Activation plotted for each cell number against the indicated concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

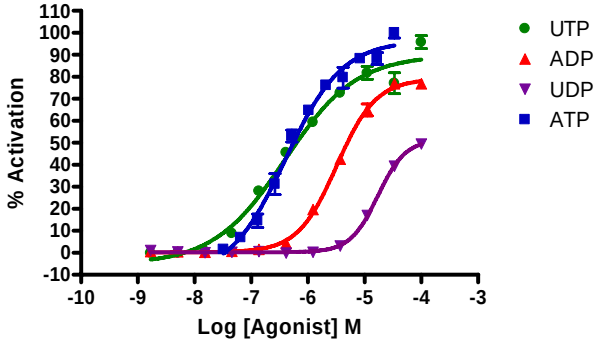
Figure 3 — GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to Suramin



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells were plated 16-20 hours prior to assay at 10,000 cells per well in a black-walled, clear bottom 384-well plate. A dilution series of Suramin (Sigma #S2671) in the presence of 0.5% DMSO was then added to the cells. The cells were incubated at 37ºC with 5% CO_2 for 30 min. ATP (Sigma #A7699) was added to the plate at the EC_{80} concentration of 3.0 µM. Cells were incubated for 4.5 hours and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Inhibition shown plotted against the concentrations of the antagonists. (n=16 for each data point).

Agonist 2nd Messenger Dose Response

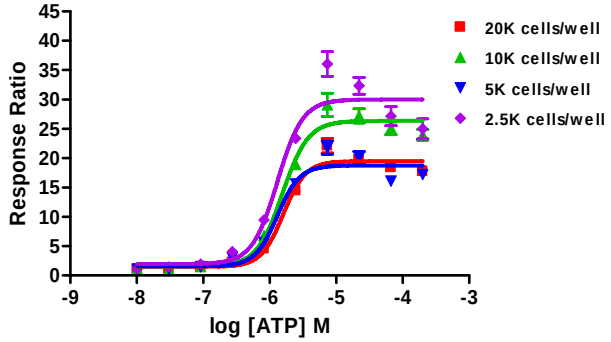
Figure 4 – GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP, UTP, ADP, and UDP as determined by measurement of intracellular Ca^{2+} using Fluo4



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were loaded with Fluo4-AM and incubated at 37°C for 60 minutes followed by a 30 minutes at room temperature. Cells were stimulated with a dilution series of adenosine-5'-triphosphate (Sigma #A7699), uridine-5'-triphosphate (Sigma #U1006), adenosine-diphosphate (Sigma #A2754), and uridine-diphosphate (Sigma #U4125) with the relative fluorescence determined by the FDSS every second for 180 seconds. The maximum minus minimum relative fluorescent values were obtained and the % Activation plotted for each concentration of agonist (n=8 for each data point).

Assay Performance with Variable Cell Number

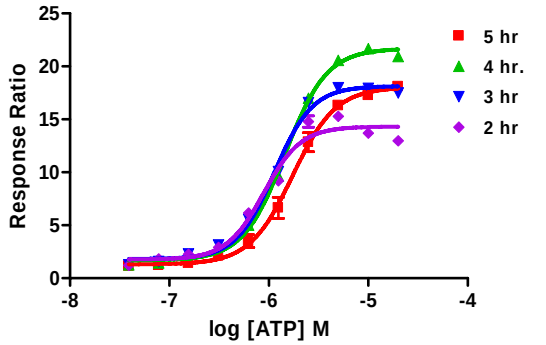
Figure 5 – GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells were plated at 2500, 5000, 10000, or 20,000 cells/well in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were then stimulated with a dilution series of ATP (Sigma #A7699) in the presence of 0.1% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained and the Response Ratios plotted for each cell number against the indicated concentrations of ATP (n=8 for each data point).

Assay Performance with Variable Stimulation Time

Figure 6 – GeneBLAzer® 2RY11-NFAT-*bla* CHO-K1 dose response to ATP with 2, 3, 4 and 5 hr stimulation times

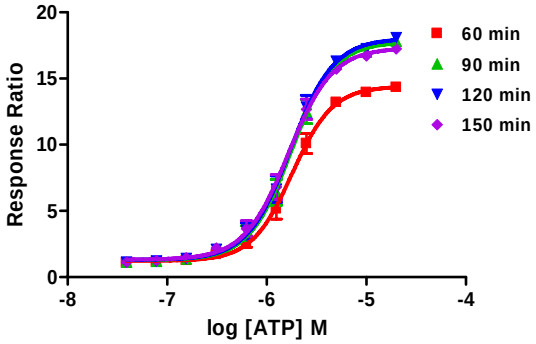


GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were then stimulated with a dilution series of ATP (Sigma cat# A7699) for 2, 3, 4, or 5 hrs in the presence of 0.5% DMSO. Cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each stimulation time against the indicated concentrations of ATP (n=8 for each data point).

References

Assay Performance with Variable Substrate Loading Times

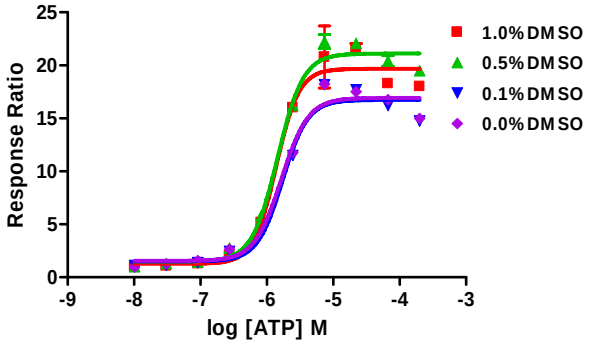
Figure 7 – GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP with 1, 1.5, 2, and 2.5 hour substrate loading times.



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were then stimulated with a dilution series of ATP (Sigma cat#7699) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 1, 1.5, 2, or 2.5 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of ATP (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 dose response to ATP with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® P2RY11-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were then stimulated with a dilution series of ATP (Sigma #7699) for 5 hours. DMSO was added to the cells at concentrations from 0% to 1%. Cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each DMSO concentration against the indicated concentrations of ATP (n=8 for each data point).

1. Ralevic, V. and Burnstock, G. (1998) **Receptors for Purines and Pyrimidines**. *Pharmacological Reviews*, **50(3)**, 413-492.
2. Cummuni, D., *et al*. (1997) **Cloning of a human purinergic PRY receptor coupled to phospholipase C and adenylyl cyclase**. *Journal of Biological Chemistry*, **272(51)**, 31969-31973.
3. Moore, DJ., *et al*. (2001) **Expression pattern of human P2Y receptor subtypes: a quantitative reverse transcription-polymerase chain reaction study**. *Biochim. Biophys. Acta*., **1521**, 107-119.
4. Communi, D., *et al*. (2000) **Rapid up-regulation of P2Y messangers during granulocytic differentiation of HL-60 cells**. *FEBS Letters*, **475**, 39-42.
5. Wilkin, F., *et al.* (2001) **The P2Y11 receptor mediates the ATP-induced maturation of human monocyte-derived dendritic cells**. *Journal of Immunology*, **166**, 7172-7177.
6. Communi, D., *et al*. (1999) **Pharmacological characterization of the human P2Y11 receptor**. *British Journal of Pharmacology*, **128**, 1199-1206.
7. Qi, AD., *et al.* (2001) **Differential coupling of the human P2Y11 receptor to phospholipase C and adenylyl cyclase**. *British Journal of Pharmacology*, **132**, 318-326.
8. White, PJ., *et al*. (2003) **Characterization of a Ca2+ response to both UTP and ATP at human P2Y11 receptors: evidence for agonist-specific signaling**. *Molecular Pharmacology*, **63**, 1356-1363.