GeneBLAzer® P2RY2 CHO-K1 DA Assay Kit

GeneBLAzer® P2RY2 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1337 and K1722

Cell Line Descriptions

GeneBLAzer® P2RY2 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 cells contain the human purinergic receptor P2, G protein-coupled, 2 (P2RY2) receptor (Accession # BC012104) stably integrated into the CellSensor® NFAT-*bla* CHO-K1 cell line. CellSensor® NFAT-*bla* CHO-K1 (Cat No. K1534) cells 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 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® P2RY2 CHO-K1 DA cells and GeneBLAzer® P2RY2-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® P2RY2-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).

P2RY2 expression has been identified in smooth muscle, skeletal muscle, heart, spleen, lymphocytes, macrophages, bone marrow, lung, intestine, placenta, brain, kidney, and liver (2). P2RY2 has been implicated in the induction of Cl^- secretion in airway epithelial (3), increase in coronary blood flow by vasodilation (4), and epidermal homeostasis (5).

P2RY2 stimulation is mediated by nucleotide triphosphates (ATP and UTP), with minimal to no activity with the nucleotide diphosphates (ADP and UDP) (1, 6). The P2RY2 receptor is coupled to the Gq/11 subunit. In the P2RY2-NFAT-bla CHO-K1 cell line the beta-lactamase gene was linked to an NFAT response element. When intracellular calcium levels rise the NFAT is activated inducing transcription of the beta-lactamase gene.

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}	150 nM	226 nM
Z'-factor	0.80	0.75

Recommended cell no.	= 10 K 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}	= 63 nM
ADP EC_{50}	= 2 µM
UDP EC_{50}	= 4 µM

3. **Antagonist dose response**

Suramin IC_{50}	= 123 µM

4. **Agonist 2nd messenger dose response**

ATP EC_{50}	= 388 nM
UTP EC_{50}	= 166 nM
ADP EC_{50}	= 9.1 µM
UDP EC_{50}	= 11.3 µM

Assay Testing Summary

5. **Assay performance with variable cell number**

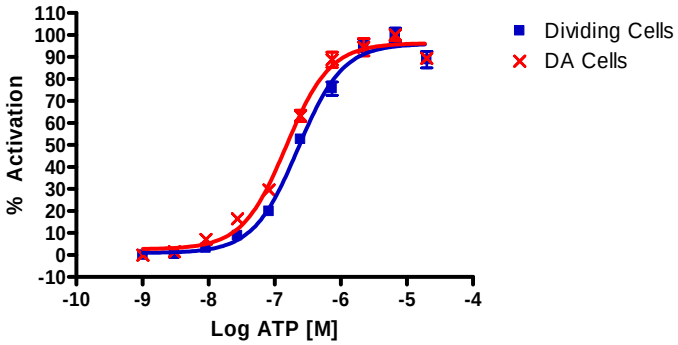
6. **Assay performance with variable stimulation time**

7. **Assay performance with variable substrate loading time**

8. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

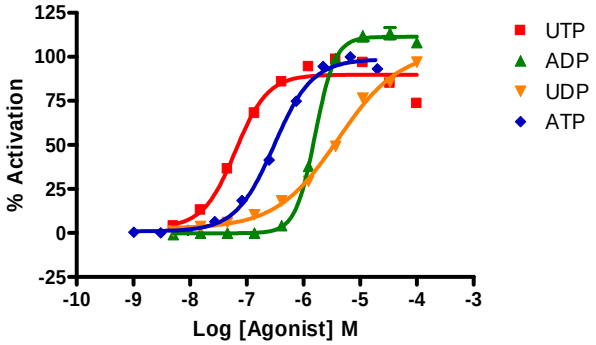
Figure 1 — GeneBLAzer® P2RY2 CHO-K1 DA and GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP under optimized conditions



GeneBLAzer® P2RY2 CHO-K1 DA cells and GeneBLAzer® P2RY2-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 the % Activation plotted for each replicate against the concentrations of ATP (n=6 for each data point).

Alternate Agonist Dose Response

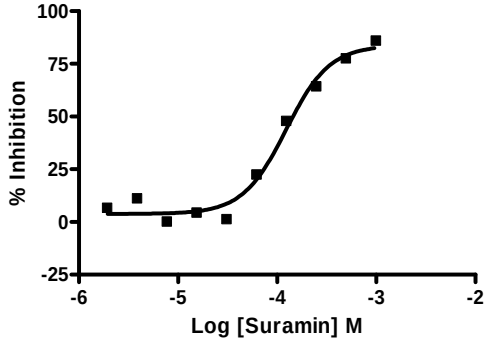
Figure 2 — GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to various agonists



GeneBLAzer® P2RY2-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 #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 is plotted for each cell number against the concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

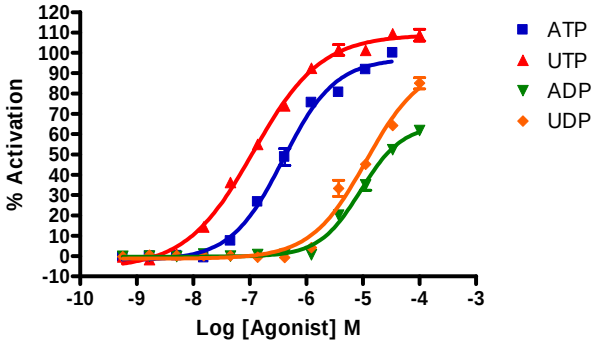
Figure 3 — GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to Suramin



GeneBLAzer® P2RY2-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 1.0% DMSO was 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 700 nM. 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 plotted against the indicated concentrations of the antagonist. (n=16 for each data point).

Agonist Dose Response Using Fluo4-NW

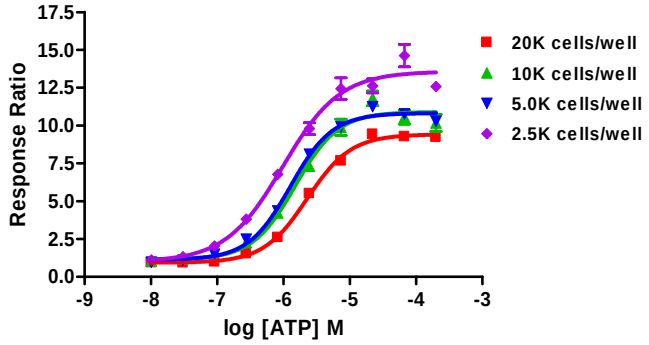
Figure 4 — GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP, UTP, ADP and UDP as determined by the measurement of intracellular Ca^{2+} by Fluo4-NW



GeneBLAzer® P2RY2-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 then 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 determined and % Activation plotted for each concentration of agonist (n=8 for each data point).

Assay Performance with Variable Cell Number

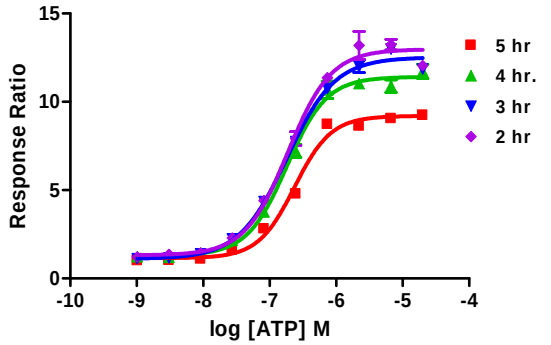
Figure 5 – GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a black-walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were 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

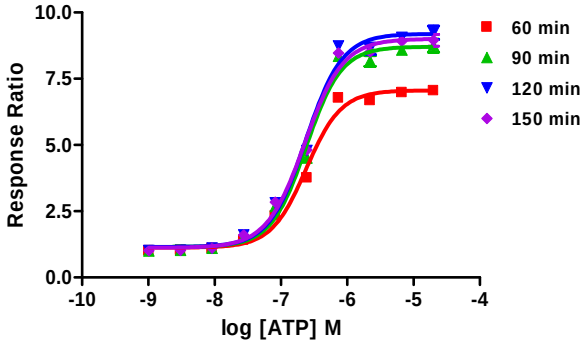
Figure 6 – GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP with 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® P2RY2-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 stimulated with a dilution series of ATP (Sigma #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).

Assay Performance with Variable Substrate Loading Times

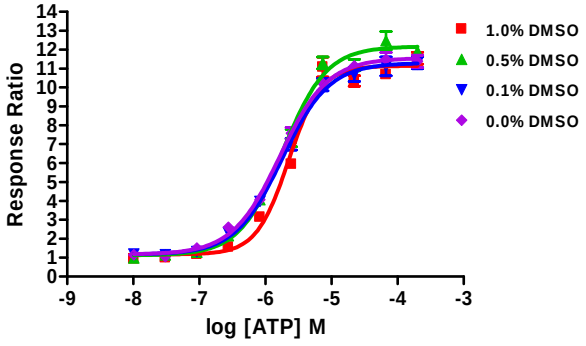
Figure 7 – GeneBLAzer® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP d with 1, 1.5, 2, and 2.5 hour substrate loading times.



GeneBLAzer® P2RY2-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 stimulated with a dilution series of ATP (Sigma #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® P2RY2-NFAT-*bla* CHO-K1 dose response to ATP with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® P2RY2-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 stimulated with a dilution series of ATP (Sigma #7699) for 5 hours. DMSO was added to the cells at concentrations ranging 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).

References

1. Ralevic, V. and Burnstock, G. (1998) **Receptors for Purines and Pyrimidines**. *Pharmacological Reviews*, **50(3)**, 413-492.

2. 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.

3. Ghanenm, E., *et al*. (2005) **The role of epithelial P2Y(2) and P2Y(4) receptors in the regulation of intestinal chloride secretion**. *British Journal of Pharmacology*, **146**, 364-369.

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