GeneBLAzer® P2RY6 CHO-K1 DA Assay Kit

GeneBLAzer® P2RY6 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1327 and K1717

Cell Line Descriptions

GeneBLAzer® P2RY6 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells contain the human purinergic receptor P2, G protein-coupled, 6 (P2RY6) receptor (Accession # NM_004154) 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 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® P2RY6 CHO-K1 DA cells and GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Uridine 5'-diphosphate (UDP); (Figure 1). In addition, GeneBLAzer® P2RY6-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).

P2RY6 has been found in a variety of rat tissues including the placenta, spleen, thymus, lung, stomach, intestine, mesentery, heart, and aorta (4). P2RY6 is activated by UDP, which functions as a full agonist. P2RY6 is activated either weakly or not at all by additional extracellular nucleotides such as UTP, ATP, and ADP (2). P2RY6 has been implicated to be involved in ion transport (5), vasodilatation (6, 7), and contraction of smooth muscle (7).

The P2RY6 receptor is coupled to the Gq/11 subunit. Agonist binding to the P2RY6 receptor will activate phospholipase C, which will initiate the phosphatidylinositol turnover response, leading to the inositol triphosphate-mediated release of calcium from the endoplasmic reticulum and to diacyglycerol activation of protein kinase C.

Validation Summary

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

1. **UDP agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	15 nM	18 nM
Z'-factor	0.89	0.86

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1.0%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 3 µM

2. **Alternate agonist dose response**

ADP EC_{50} = 5.2 µM

3. **Antagonist dose response**

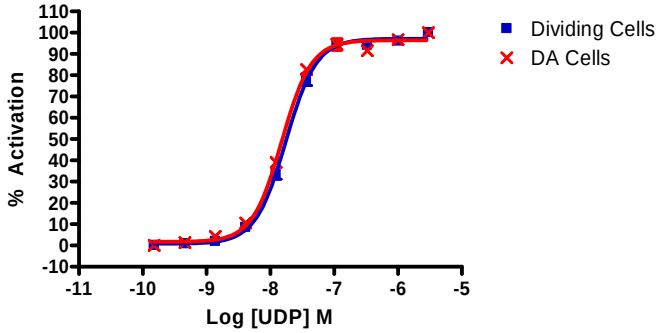
MRS2578 IC_{50} = 2.7 µM

Assay Testing Summary

4. **Assay performance with variable cell number**
5. **Assay performance with variable stimulation time**
6. **Assay performance with variable substrate loading time**
7. **Assay performance with variable DMSO concentration**
8. **Assay performance with dialyzed and charcoal treated FBS**
9. **Assay performance with variable concentrations of charcoal treated FBS**

Primary Agonist Dose Response

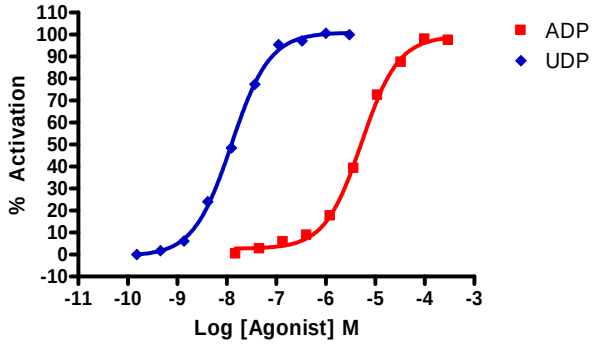
Figure 1 — GeneBLAzer® P2RY6 CHO-K1 DA and GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP under optimized conditions



GeneBLAzer® P2RY6 CHO-K1 DA cells and GeneBLAzer® P2RY6-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 UDP 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 UDP (n=6 for each data point).

Alternate Agonist Dose Response

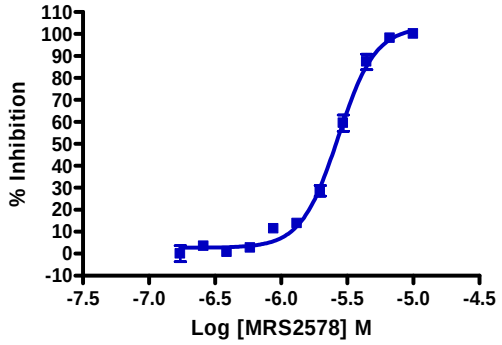
Figure 2 — GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP and ADP



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells were plated at 10,000 cells/well in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of UDP (Sigma #U4125) and ADP (Sigma #A2754) 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 against the indicated concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

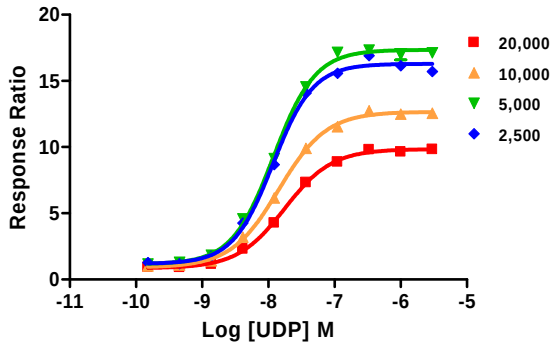
Figure 3 — GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to MRS 2578



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells were plated 16-20 hours prior to assay at 10,000 cells per well in a 384-well format. Dilution series of MRS 2578 (Sigma #M0319) in the presence of 0.5% DMSO was added to the cells. The cells were incubated at 37ºC with 5% CO_2 for 30 min. UDP (Sigma #U4125) was added to the plate at the EC_{80} concentration of 50 nM. Cells were incubated for 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 concentrations of MRS 2578. (n=8 for each data point).

Assay Performance with Variable Cell Number

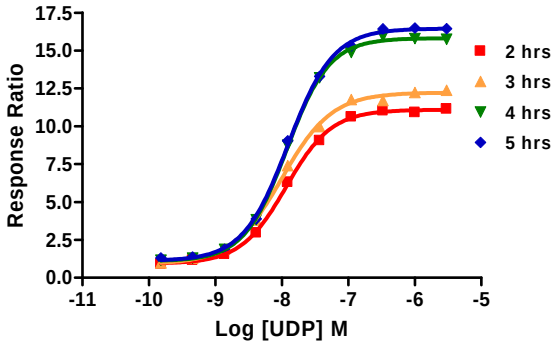
Figure 4 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format in assay media, and incubated at 37°C for 16-20 hours. Cells were stimulated with a dilution series of UDP (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 Response Ratios were plotted for each cell number against the concentrations of UDP (n=8 for each data point).

Assay Performance with Variable Stimulation Time

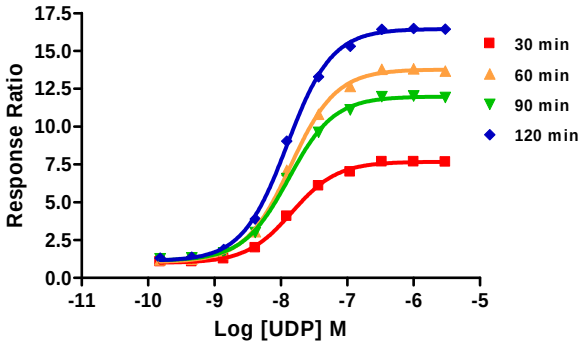
Figure 5 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP with 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format in assay media, and incubated at 37°C for 16-20 hours. Cells were stimulated with a dilution series of UDP (Sigma #U4125) 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 were plotted for each stimulation time against the concentrations of UDP (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

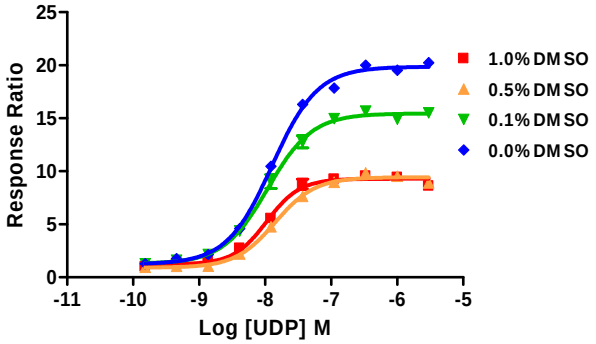
Figure 6 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP with 0.5, 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format in assay media, and incubated at 37°C for 16-20 hours. Cells were stimulated with a dilution series of UDP (Sigma #U4125) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 0.5, 1, 1.5, or 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 were plotted for each substrate loading time against the concentrations of UDP (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

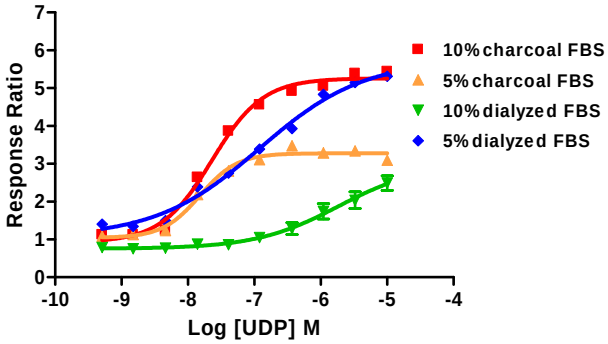
Figure 7 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format in assay media, and incubated at 37°C for 16-20 hours. DMSO was added to the cells at concentrations from 0% to 1%. Cells were stimulated with a dilution series of UDP (Sigma #U4125) for 5 hours. 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 were plotted for each DMSO concentration against the concentrations of UDP (n=8 for each data point).

Assay performance with Dialyzed vs. Charcoal Treated FBS

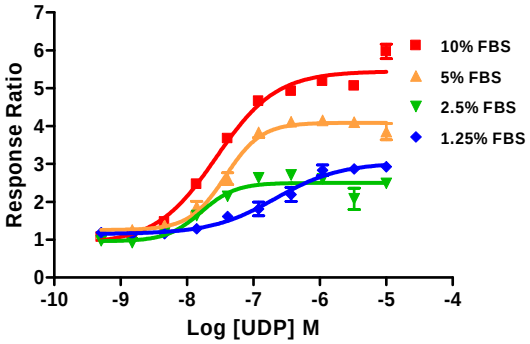
Figure 8 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP using dialyzed or charcoal treated FBS in the assay media.



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format in assay media supplemented with either 10% or 5% dialyzed FBS or charcoal treated FBS. Cells were incubated at 37°C for 16-20 hours. Cells were stimulated with a dilution series of UDP (Sigma #U4125) for 5 hours 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 were plotted for each FBS concentration against the concentrations of UDP (n=8 for each data point).

Assay Performance with Variable Concentrations of Charcoal Treated FBS

Figure 9 – GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 dose response to UDP with varying concentrations of charcoal treated FBS in the assay media.



GeneBLAzer® P2RY6-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format in assay media supplemented with 10%, 5%, 2.5%, or 1.25% charcoal treated FBS. Cells were incubated at 37°C for 16-20 hours. Cells were stimulated with a dilution series of UDP (Sigma #U4125) for 5 hours 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 were plotted for each FBS concentration against the concentrations of UDP (n=8 for each data point).

References

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