GeneBLAzer® PTGDR CHO-K1 DA Assay Kit

GeneBLAzer® PTGDR CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1373 and K1739

Cell Line Descriptions

GeneBLAzer® PTGDR CHO-K1 DA (Division Arrested) cells and GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells contain the human Prostanoid (PTGDR) receptor (Accession #NM_000953.2) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase (*bla*) reporter gene under control of the Cyclic AMP Response Element (CRE). Division Arrested (DA) cells are available in 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® PTGDR CHO-K1 DA cells and GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Prostaglandin D2, (Figure 1). In addition, PTGDR-CRE-*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

Prostanoids are the metabolites of arachidonic acid by cyclooxygenase and include prostaglandins (PGs) and thromboxanes (TXs). There are five primary types of prostanoids including PGE_2, PGD_2, $PGF_{2\alpha}$. PGI_2, and TxA_2. Following cell stimulation, prostanoids are synthesized, released, and exert their action on cells in the vicinity of their release. The receptors that mediate the actions of the prostanoids belong to the G protein-coupled receptor (GPCR) superfamily and are termed P receptors with a preceding letter to designate the natural prostanoid to which each receptor is most sensitive: EP, FP, IP, TP, and DP. The EP class has four receptors named EP1, EP2, EP3, and EP4 based upon the sensitivity to various agonist and antagonists. Each of these receptors belongs to the rhodopsin-type sub-family of GPCRs.

The prostaglandin D2 receptor (PTGDR), also known as DP, is a Gs coupled receptor which activates adenylate cyclase (1-3). PTGDR receptors are located in vascular smooth muscle cells and blood platelets, and are also believed to be expressed in the brain, where they may be involved in the regulation of sleep (4). In addition, knockout studies in mice suggest that PTGDR may be involved in allergic and asthmatic responses (5). Potent agonists and antagonists for PTGDR have been identified (6-9). Futher information on prostanoid receptors can be obtained from review articles (10-11).

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Prostaglandin D_2 agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	0.4 nM	0.3 nM
Z'-factor	0.82	0.82

Optimum cell no. = 20K cells/well
Optimum [DMSO] = up to 1%
Stimulation Time = 5 hours
Max. [Stimulation] = 14 nM

2. **Alternate agonist dose response**

BW245C EC_{50} = 22 pM
Prostaglandin E_2 EC_{50} = 13 nM

3. **Antagonist dose response**
See antagonist dose response section

4. **Agonist 2nd Messenger Dose Response**
Prostaglandin D_2 EC_{50} = 2 nM

Assay Testing Summary

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

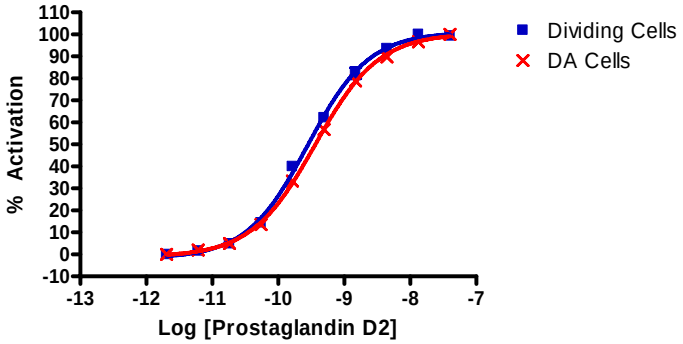
6. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

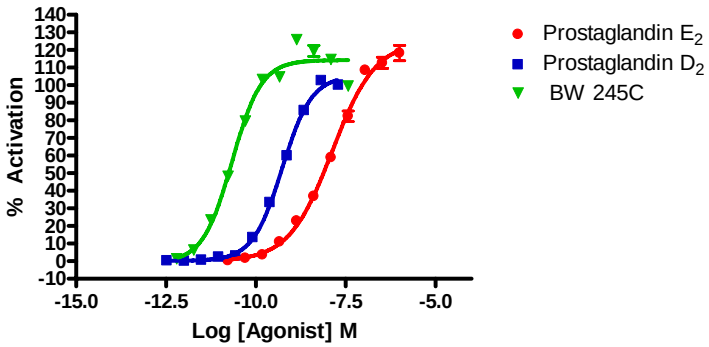
Figure 1 — GeneBLAzer® PTGDR CHO-K1 DA and PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D2 under optimized conditions



GeneBLAzer® PTGDR CHO-K1 DA cells and GeneBLAzer® PTGDR-CRE-*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 Prostaglandin D2 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 Prostaglandin D2 (n=6 for each data point).

Alternate Agonist Dose Response

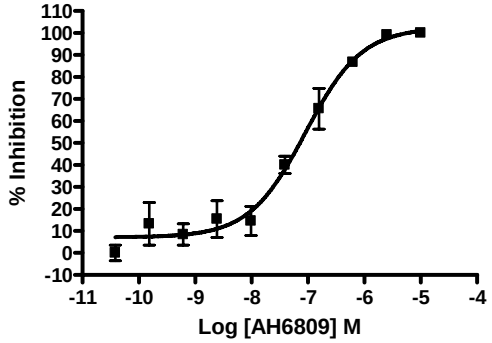
Figure 2 — GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D_2, BW 245C, and Prostaglandin E_2



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells (20,000 cells/well) were plated the day before the assay in a 384-well format. Cells were stimulated with either Prostaglandin D_2 (Sigma #P5172), BW 245C (Sigma #B9305), or Prostaglandin E_2 (Sigma #P5640) over the indicated concentration range 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 Ratios plotted against the indicated concentrations of the agonists (n= 8 for each data point). The data shows the correct rank order potency.

Antagonist Dose Response

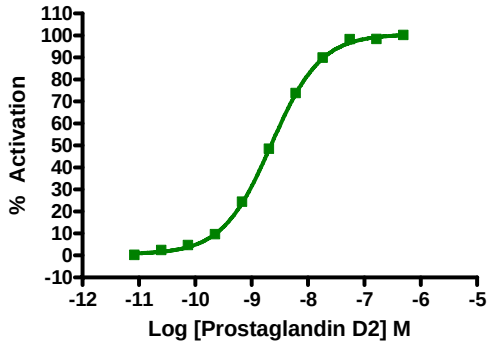
Figure 3 — GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to AH6809



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells (20,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with AH6809 (Sigma #A1221), and incubated at 37 degrees C for 30 min., followed by 600 pM Prostaglandin D_2 agonist stimulation for 5 hours in 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 fluorescence plate reader and the Ratios plotted against the indicated concentrations of the antagonist (n=2 for each data point).

Agonist 2nd Messenger Response

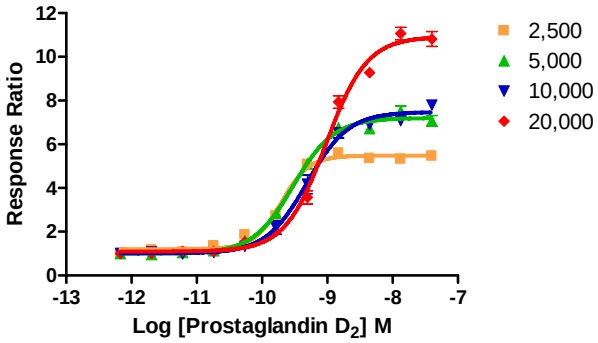
Figure 4— GeneBLAzer® PTGDR-CRE-*bla* CHO-k1 2nd messenger dose response to Prostaglandin D2 under optimized conditions



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells were tested for a response to Prostaglandin D2 with a TR-FRET cAMP assay.

Assay Performance with Variable Cell Number

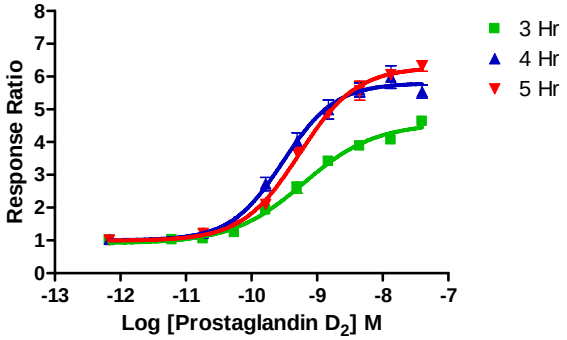
Figure 5— GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D_2 with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells were plated the day before the assay at 2,500, 5,000, 10,000 or 20,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with Prostaglandin D_2 (Sigma #P5172) 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of Carbachol (n=8 for each data point).

Assay performance with Variable Stimulation Time

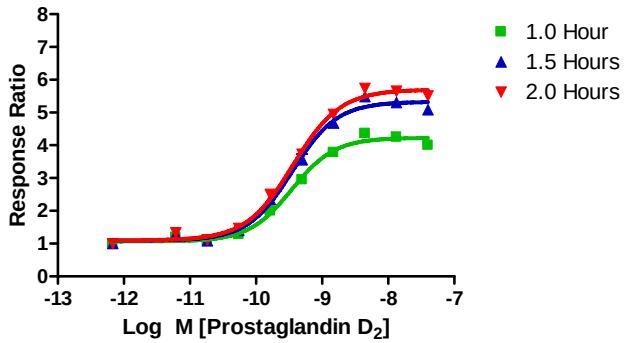
Figure 6 – GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D_2 with 3, 4, and 5 hour stimulation times



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells (20,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. Prostaglandin D_2 (Sigma #P5172) was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs in 0.5% DMSO and 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 fluorescence plate reader and the Response Ratios plotted (n=8 for each data point).

Assay performance with Variable Substrate Loading Time

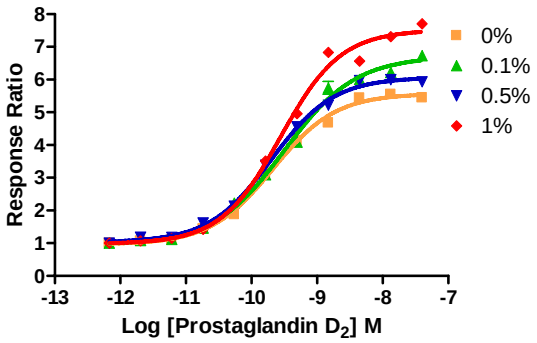
Figure 7 – GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D_2 with 1, 1.5, and 2 hour loading times



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells were plated at 20,000 cells/well in a 384-well format the day before the assay. On the day of the assay, cells were stimulated with Prostaglandin D_2 (Sigma #P5172) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5, and 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of Prostaglandin D_2 (n=8 for each data point).

Assay Performance with variable DMSO concentration

Figure 8 – GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 dose response to Prostaglandin D_2 with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® PTGDR-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Carbachol (Sigma #21760) was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 5 hrs with agonist 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 Response Ratios are shown plotted for each DMSO concentration against the indicated concentrations of carbachol (n=8 for each data point).

References

1. Alvarez, R., Taylor, A., Fazzari, J.J. and Jacobs, J.R. (1981) **Regulation of cyclic AMP metabolism in human platelets. Sequential activation of adenylate cyclase and cyclic AMP phosphodiesterase by prostaglandins.** *Mol. Pharmacol.,* **20,** 302-309.

2. Trist, D.G., Collins, B.A., Wood, J., Kelly, M.G. and Roberson, A.D. (1989) **The antagonism by BW A868C of PGD2 and BW 245C activation of human platelet adenylate cyclase.** *Br. J. Pharmacol,* **96,** 301-306.

3. Ito, S., Negishi, M., Sugama, K., Okuda-Ashitaka, K. and Hayaishi, O. (1990) **Signal transduction coupled to prostaglandin D_2.** *Adv. Prostaglandin Thromboxane Leukotriene Res.,* **21A,** 371-374.

4. Hayaishi, O., Matsumura, H., Onoe, H., Koyama, Y. and Watanabe, Y. (1991) **Sleep-wake regulation by PGD2 and E2.** *Adv. Prostaglandin Thromboxane Leukotriene Res.,* **21,** 723-726.

5. Matsuoka, T., et. al. (2000) **Prostaglandin D2 as a mediator of allergic asthma.** *Science*, **287,** 2013-2017.

6. Alvarez, R., Eglen, R.M., Chang, L.F., Bruno, J.J., Artis, D.R., Kluge, A.F. and Whiting, R.L. (1991) **Stimulation of prostaglandin D_2 receptors on human platelets by analogs of prostacyclin.** *Prostaglandins,* **42,** 105-119.

7. Thierauch, K-H., Sturzebecher, C. St., Schillinger, E., Rehwinkel, H., Raduchel, B., Skuballa, W. and Vorbruggen, H. (1988) **Stable 9- or 11-halogen-15-cyclohexyl-prostaglandins with high affinity to the PGD_2 receptor.** *Prostaglandins,* **35, 853-868.**

8. Giles, H., Leff, P., Bolofo, M.L., Kelly, M.G. and Robertson, A.D. (1989) **The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective, and potent competitive antagonist.** *Br. J. Pharmacol.,* **96,** 291-300.

9. Keery, R.J. and Lumley, P. (1988) **AH6809, a prostaglandin DP-receptor blocking drug on human platelets.** *Br. J. Pharmacol.,* **94,** 745-754.

10. Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999) **Prostanoid receptors: structures, properties, and functions.** *Physiol. Rev.,* **79,** 1193-1226.

11. Breyer, R.M., Bagadassarian, C.K., Myers, S.A. and Breyer, M.D. (2001) **Prostanoid receptors: subtypes and signaling.** *Annu. Rev. Pharmacol. Toxicol.,* **41,** 661-690.