GeneBLAzer® VPAC2 CHO-K1 DA Assay Kit

GeneBLAzer® VPAC2 CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1305 and K1706

Cell Line Descriptions

GeneBLAzer® VPAC2 CHO-K1 DA (Division Arrested) cells and GeneBLAzer®VPAC2-CRE-*bla* CHO-K1 cells contain the human Vasocative intestinal peptide/ pituitary adenylate cyclase activating polypeptide receptor 2 (VPAC2), (Accession #NM_003382) 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 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® VPAC2 CHO-K1 DA cells and GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of VIP peptide (Figure 1). In addition, GeneBLAzer® VPAC2-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

Vasoactive Intestinal Peptide/Pituitary Adenylate Cyclase Activating Polypeptide (VPAC) receptors belong to the secretin family of receptors. VPAC1 was originally cloned in 1992 from rat lung (1) and the human form was cloned a year later from HT29 cells (2). In this same year, a closely related VPAC2 receptor was cloned from pituitary gland (3). VPAC1 and VPAC2 share 50% homology and show similar affinity for the natural peptides, VIP and PACAP (4). A specific PACAP receptor (later named PAC1) which has low affinity for VIP was also cloned in 1993 (rev in 5). VPAC receptors have been characterized in almost all mammalian tissues including brain, liver, pancreas, and intestine in concordance with the wide distribution of VIP (6).

Vasoactive Intestinal Peptide (VIP) and Pitituitary Adenylate-cyclase Activating Polypeptide (PACAP) act as agonists at the VPAC receptors and do not clearly discriminate between the two forms of VPAC receptors (7). Vasoactive Intestinal Peptide (VIP) is a 28-amino acid neurotransmitting/ neuromodulatory peptide that is widely distributed in both the central nervous system and peripheral tissues (7). VIP plays an important role in the perception of pain, suppression of inflammation, immunomodulation, exocrine secretions, hormone release, muscle relaxation, metabolism, and also serves as a growth regulator for fetuses (8-13). VPAC2 receptors have been implicated in a wide range of disorders, including rheumatoid arthritis (14), Crohn´s disease (15). VPAC2 has also been implicated in pancreatic insulin secretion and may be a Type 2 diabetes target (16).

Validation Results

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

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

	DA cells	Dividing Cells
EC_{50}	74 pM	407 pM
Z'-factor	0.69	0.68

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim. Time	= 3 hours
Max. [Stimulation]	= 64 nM

2. **Alternate agonist dose response**

PACAP	EC_{50}	= 56pM
VPAC2	EC_{50}	= 77pM

3. **Antagonist Dose Response**
   There are no known VPAC2 antagonists at the time of publication of this document

4. **Agonist 2nd Messenger Dose Response**

VIP	EC_{50}	= 259 pM

Assay Performance Summary

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

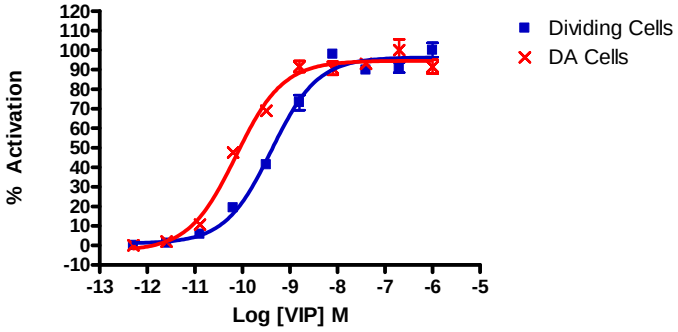
6. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

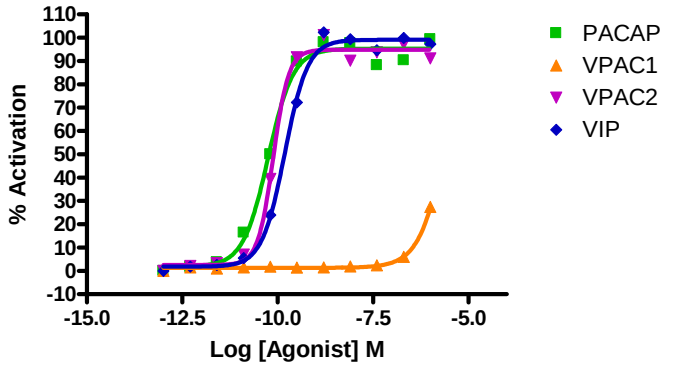
Figure 1 — GeneBLAzer® VPAC2 CHO-K1 DA and VPAC2-CRE-*bla* CHO-K1 dose response to VIP under optimized conditions



GeneBLAzer® VPAC2 CHO-K1 DA cells and VPAC2-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 VIP 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 VIP (n=6 for each data point).

Alternate Agonist Dose Response

Figure 2 — GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 dose response to PACAP, VPAC1, VPAC2, and VIP.



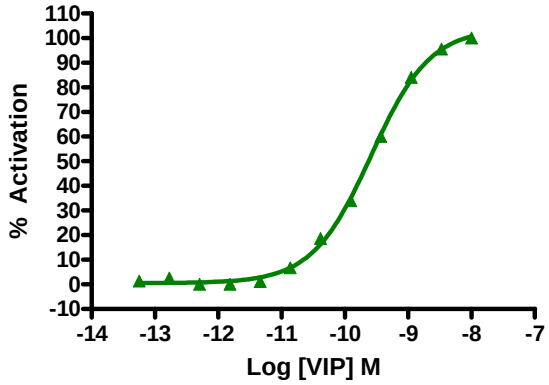
GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Cells were stimulated with either VIP (Anaspec #22872), VPAC2 agonist (Phoenix #064-28), PACAP (Anaspec #22527), or VPAC1 agonist (Phoenix #064-24) over the indicated concentration range in the presence of 0.5% DMSO for 3 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 is shown plotted against the indicated concentrations of agonist. The data shows the correct rank order potency and selectivity for PACAP, VPAC2, VPAC1, and VIP agonists.

Antagonist Dose Response

There are no known VPAC2 antagonists available for testing at the time of publication of this document

Agonist 2nd Messenger Response

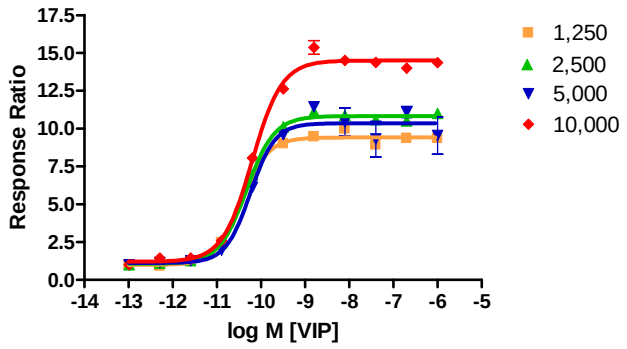
Figure 3— GeneBLAzer® VPAC2-CRE-*bla* CHO-k1 2nd messenger dose response to VIP under optimized conditions



GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells were tested for a response to VIP with a TR-FRET cAMP assay.

Assay Performance with Variable Cell Number

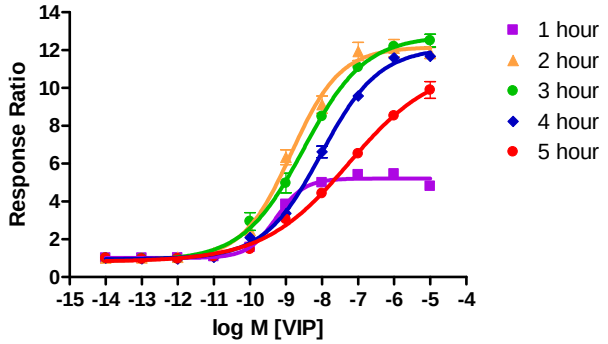
Figure 4— GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 dose response using 1.25, 2.5, 5 and 10K cells/well



GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells were plated the day before the assay at 1250, 2500, 5000 or 10000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with VIP (Anaspec #22872) 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 VIP(n=8 for each data point).

Assay Performance with Variable Stimulation Time

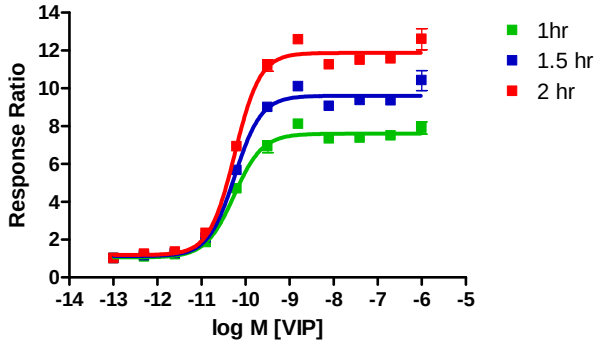
Figure 5 – GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 dose response using 1, 2, 3, 4, and 5 hour stimulation times



GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. VIP (Anaspec #22872) was then added to the plate over the indicated concentration range for 1, 2, 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 for each stimulation time against the indicated concentrations of VIP(n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

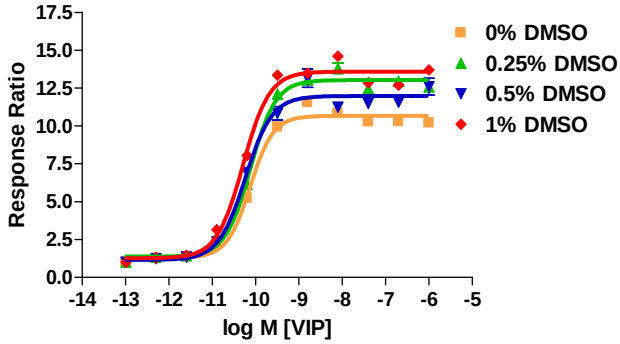
Figure 6 – GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 dose response using 1, 1.5 and 2hr substrate loading times



GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells were plated the day before the assay at 10000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with VIP (Anaspec #22872) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 1, 1.5 or 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 VIP(n=8 for each data point).

Assay Performance with variable DMSO concentration

Figure 7 – GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 dose response using 0, 0.25, 0.5 and 1% DMSO



GeneBLAzer® VPAC2-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. VIP (Anaspec #22872) 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 3 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 VIP(n=8 for each data point).

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