GeneBLAzer® VPAC1 CHO-K1 DA Assay Kit

GeneBLAzer® VPAC1 CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1303 and K1705

Cell Line Descriptions

GeneBLAzer® VPAC1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells contain the human Vasoactive intestinal peptide/pituitary adenylate cyclase activating polypeptide receptor 1 (VPAC1), (Accession # NM_004624) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1129) 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® GeneBLAzer® VPAC1 CHO-K1 DA cells and VPAC1-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of VIP peptide (Figure 1). GeneBLAzer® GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells have also 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). PACAP-27 shares 68% sequence identity with VIP, and it has been shown that VPAC receptors display similar affinity for VIP and PACAP.

Recently, VPAC1 has been implicated in Parkinson's Disease (14, 15). A murine model for Parkinson's Disease based upon treatment with the neurotoxin MPTP produces irreversible clinical, biochemical, and neuropathological effects which mimic the effects of Parkinson's Disease (16,17).

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}	62 pM	64 pM
Z'-factor	0.88	0.78

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim. Time	= 3 hours
Max. [Stimulation]	= ~8nM

2. Alternate agonist dose response

VPAC1 agonist	EC_{50}	= 83 pM
VPAC2 agonist	EC_{50}	= 18 nM
PACAP	EC_{50}	= 29 pM

3. Antagonist dose response

VPAC1 antagonist	IC_{50}	= 2 nM

4. 2nd Messenger Dose Response

VIP EC_{50}	= 21 pM

Assay Performance with Variable Conditions

5. Assay performance with variable cell number

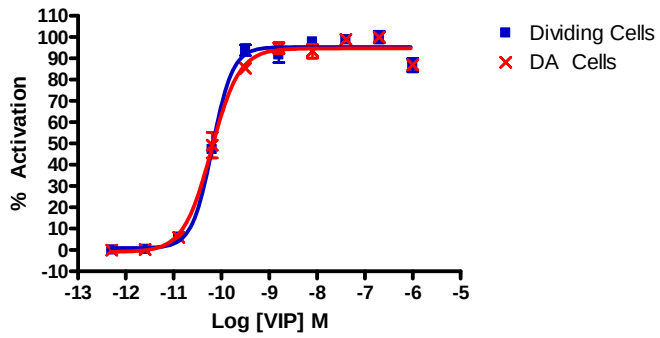
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

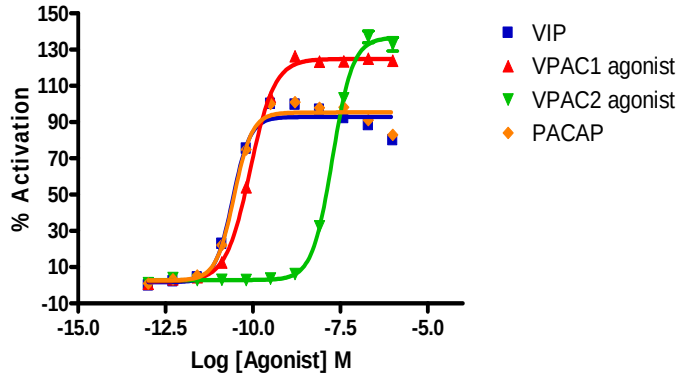
Figure 1 — GeneBLAzer® VPAC1 CHO-K1 DA and GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response to VIP peptide under optimized conditions



GeneBLAzer® VPAC1 CHO-K1 DA cells and GeneBLAzer® VPAC1-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 Peptide 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 peptide (n=6 for each data point).

Alternate Agonists Dose Response

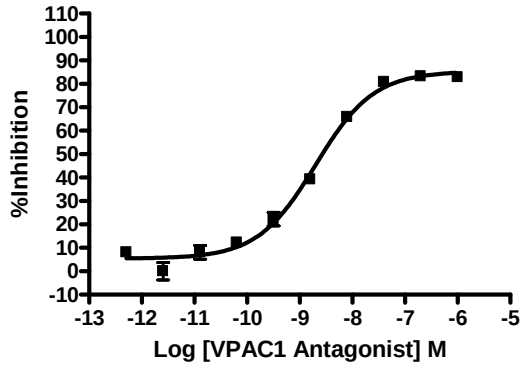
Figure 2 — GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response to VIP, VPAC1 agonist, VPAC2 agonist and PACAP



GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either VIP (Anaspec #22872), VPAC1 agonist (Phoenix #064-24), VPAC2 agonist (Phoenix #064-28), or PACAP (Anaspec 22527) 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 (n=8 for each data point). The data shows the correct rank order potency for these agonists.

Antagonist Dose Response

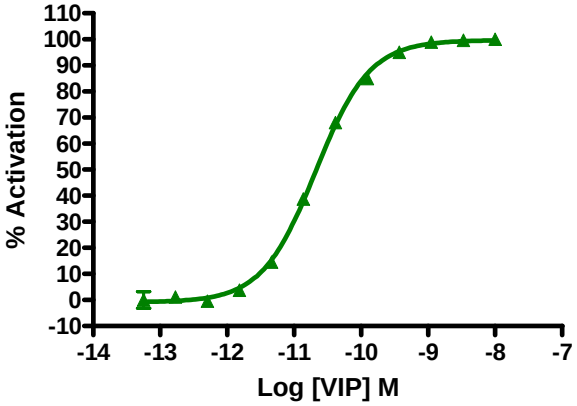
Figure 3 — GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 Dose Response to VPAC1 antagonist



GeneBLAzer® VPAC1-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 in. Cells were treated with VPAC1 Antagonist (Phoenix Pharmaceuticals Cat. #064-25) and incubated at 37 degrees C for 30 min., followed by 64pM VIP stimulation for 3 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 % Inhibition is shown plotted against the indicated concentrations of antagonist (n=8 for each data point).

Agonist 2nd Messenger Response

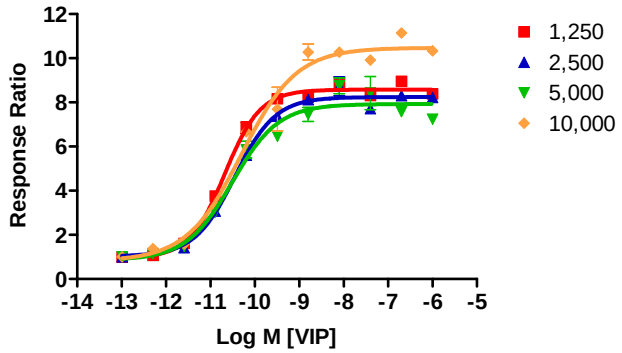
Figure 4— GeneBLAzer® VPAC1-CRE-*bla* CHO-k1 2nd messenger dose response to VIP under optimized conditions



GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells were tested for a response to VIP with a TR-FRET cAMP assay.

Assay Performance with Variable Cell Number

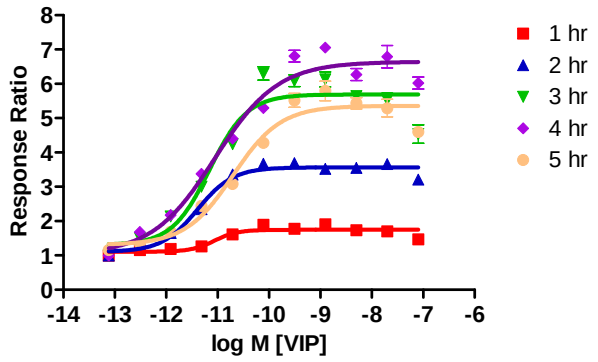
Figure 5— GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response using 1.25, 2.5, 5, and 10K cells/well



GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells were plated the day before the assay at 1,250 2,500 or 5,000 and 10,000 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 for each cell number against the indicated concentrations of VIP (n=8 for each data point).

Assay Performance with Variable Stimulation Time

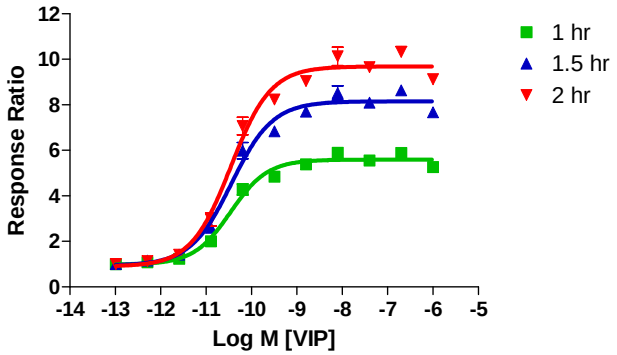
Figure 6 – GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response using 1, 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well 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 against the indicated concentrations of VIP (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

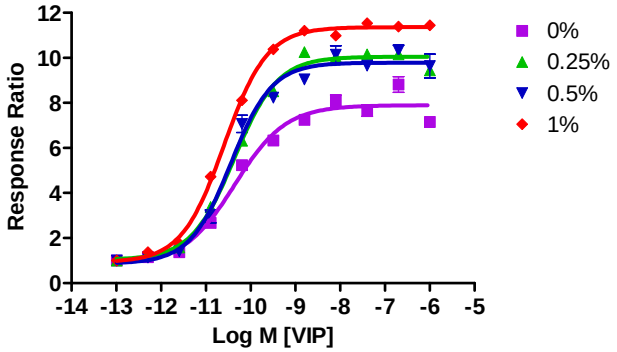
Figure 7— GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response using 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® VPAC1-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 in 0.5% DMSO for 3 hours and then loaded for 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 fluorescence plate reader and the Response Ratios for each substrate loading time plotted against the indicated concentrations of VIP (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 dose response using 0, 0.25, 0.5 and 1% DMSO



GeneBLAzer® VPAC1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. VIP (Anaspec #22872) was then added to the plate over the indicated concentration range. DMSO was added to separate wells 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 for each DMSO concentration against the indicated concentrations of VIP (n=8 for each data point).

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