GeneBLAzer® PAC1 CHO-K1 DA Cells

GeneBLAzer® PAC1-CRE-*bla* CHO-K1 Cells

Catalog Numbers –K1619 and K1525

Cell Line Descriptions

GeneBLAzer® PAC1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells contain the human Adenylate Cyclase Activating Polypeptide 1 (Pituitary) Receptor (ADCYAP1R1) (PAC1), (Accession # NM_001118) 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 cAMP response element (CRE).

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® PAC1 CHO-K1 DA cells and GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of PACAP (Figure 1). In addition, PAC1-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

Validation Summary

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

1. PACAP dose response under optimized conditions

	DA	Dividing Cells
EC_{50}	8.0 pM	22.7pM
Z'-factor	0.92	0.8

Recommended cell no. /well = 10,000
Recommended Stim. Time = 4 hrs
Max. [Stimulation] = 40 nM

2. Alternate agonist dose response

VIP EC_{50} = 34 nM
VPAC1 agonist EC_{50} = 265 nM
VPAC2 agonist EC_{50} = 95 nM

Assay Testing Summary

3. Assay performance in 2nd messenger assay.

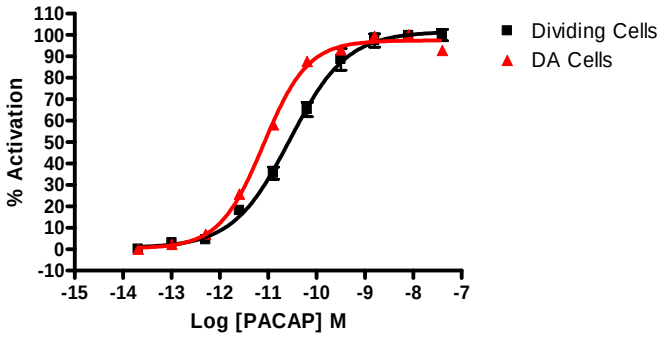
PACAP EC_{50} = 0.8 pM

4. Assay performance with variable cell number.

5. Assay performance with variable stimulation time.

Primary Agonist Dose Response

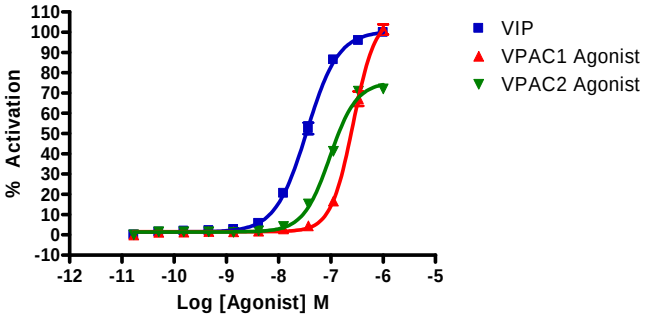
Figure 1 — GeneBLAzer® PAC1 CHO-K1 DA and GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells dose response to PACAP under optimized conditions



GeneBLAzer® PAC1 CHO-K1 DA cells and GeneBLAzer® PAC1-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 PACAP (Anaspec 22527) in the presence of 0.1% DMSO for 4 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 PACAP.

Alternate Agonist Dose Response

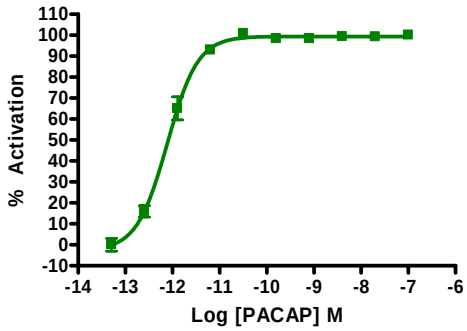
Figure 2 — GeneBLAzer® PAC1-CRE-*bla* CHO-K1 dose response to VIP and selective VPAC agonists



GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with VIP (Anaspec #22527), VPAC1 agonist (Phoenix Pharmaceuticals #064-24), or VPAC2 agonist (Phoenix Pharmaceuticals #064-28) over the indicated concentration range in the presence of 0.1% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

2nd Messenger Dose Response

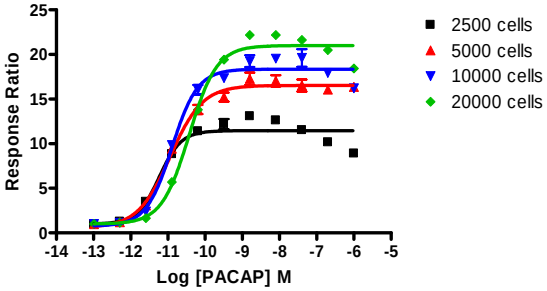
Figure 3 — GeneBLAzer® PAC1-CRE-*bla* CHO-K1 2nd messenger dose response to PACAP under optimized conditions.



GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells were tested for a response to PACAP with a TR-FRET cAMP kit.

Assay Performance with Variable Cell Number

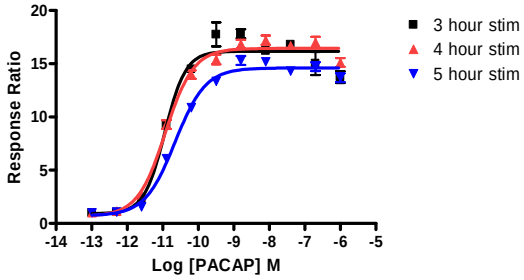
Figure 4 — GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells dose response to PACAPwith 2.5K, 5K, 10K or 20K cells/well



GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells were plated in a 384-well format at 2,500, 5,000, 10,000, or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with PACAP (Anaspec 22527) in the presence of 0.1% DMSO for 4 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 PACAP.

Assay Performance with Variable Stimulation Time

Figure 5 – GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells dose response to PACAP with 3, 4 and 5 hour stimulation times



GeneBLAzer® PAC1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. PACAP (Anaspec 22527) was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs in 0.1% DMSO. The 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 Response Ratios plotted against the indicated concentrations of PACAP.