GeneBLAzer® AVPR1A CHO-K1 DA Cells Assay Kit

GeneBLAzer® AVPR1A NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1323 and K1715

Cell Line Descriptions

GeneBLAzer® AVPR1A CHO-K1 DA (Division Arrested) cells and GeneBLAzer® AVPR1A-NFAT-*bla* CHO-K1 cells contain the human Arginine Vasopressin 1a (AVPR1A) receptor (Accession # NM_000706) 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® AVPR1A CHO-K1 DA cells and GeneBLAzer® AVPR1A-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC50 concentrations of vasopressin. In addition, GeneBLAzer® AVPR1A-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.

Target Description

The hormones vasopressin and oxytocin are synthesized in the hypothalamus. Oxytocin causes contractions in the uterus during childbirth. The main functions of arginine vasopressin (AVP) are water reabsorption by the kidney and contraction of smooth muscle in arteries. This system may also play a role in memory and learning (reviewed in 1).

The family of receptors that recognizes vasopressin and oxytocin consists of 4 members: Vasopressin receptors 1a (AVPR1a), 1b (AVPR1b), 2 (AVPR2), and the oxytocin (OT) receptor (2). AVPR1a, 1b, and the OT receptor couple to the Gq/G11 class of G proteins and trigger the release of intracellular calcium when stimulated (3-5, 7). AVPR2 receptors couple to Gs proteins and activate adenyl cyclase (6). All three receptors have high affinity for their endogenous ligand, vasopressin, but AVPR1b has less affinity for oxytocin than AVPR1a or AVPR2 (8).

The amino acid sequence of the human AVPR1a receptor is 36% and 45% identical to AVPR2 and OT receptors, respectively. Radioligand binding studies originally showed AVPR1a localization to vascular smooth muscle cells, hepatocytes, cell types in the blood, adrenal cortex, brain, reproductive organs, and kidneys (7). Studies have found AVPR1a to be involved in maintenance of blood pressure by controlling vasoconstriction (9).

Validation Results

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

1. Vasopressin dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1.1 nM	0.7 nM
Z'-Factor	0.88	0.92

Recommended cell no. = 5K cells/well
Recommended [DMSO] = up to 1%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 500 nM

2. Alternate agonist dose response

Oxytocin EC_{50} = 32.4 nM

3. Antagonist dose response

Mercapto-Cyclopentamethylene-propionyl Vasopressin IC_{50} = 3.4 nM

4. Agonist 2nd Messenger Response

Vasopressin EC_{50} = 1.9 nM

Assay Performance with Variable Conditions

5. Assay performance with variable cell number

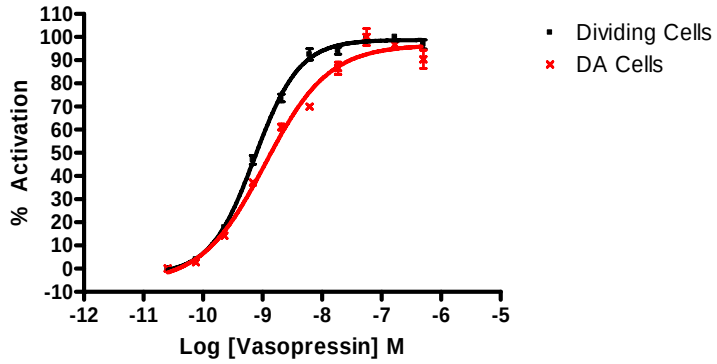
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

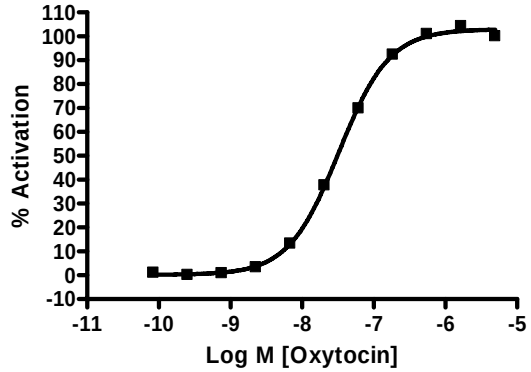
Figure 1 — GeneBLAzer® AVPR1A CHO-K1 DA and GeneBLAzer® AVPR1A-NFAT-*bla* CHO-K1 dose response to Vasopressin under optimized conditions



GeneBLAzer® AVPR1A CHO-K1 DA cells and GeneBLAzer® AVPR1A-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of vasopressin (Sigma #E2387) 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 was plotted for each replicate against the concentrations of vasopressin (n=6 for each data point).

Alternate Agonist Dose Response

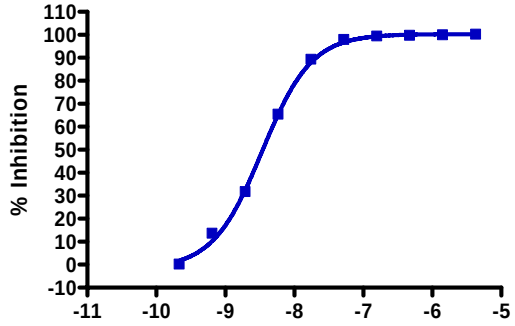
Figure 2 — GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to Oxytocin under optimized conditions



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were were plated the day before the assay in a 384-well format and stimulated with Oxytocin (Sigma #O6379) 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 % Activation plotted against the indicated concentrations of Oxytocin(n=16 for each data point).

Antagonist Dose Response

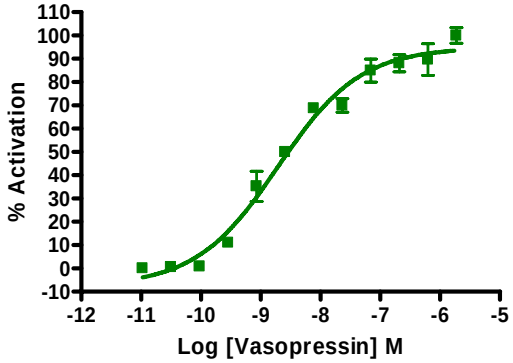
Figure 3 — GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to B-Mercapto-Cyclopentamethylene-propionyl vasopressin under optimized conditions



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-24 hours. Cells were then treated with a vasopressin derivative (Sigma #V2255) over the indicated concentration range in the presence of 0.5% DMSO for 30 minutes prior to stimulation with 3nM vasopressin (Phoenix Pharmaceuticals #065-10). 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 % Inhibition plotted against the indicated concentrations of vasopressin antagonist (n=16 for each data point).

Agonist 2nd Messenger Response

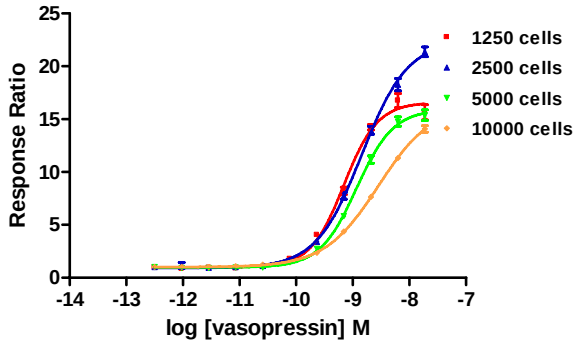
Figure 4— GeneBLAzer® AVPR1a-NFAT-*bla* CHO-k1 2nd messenger dose response to Vasopressin under optimized conditions



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to Vasopressin.

Assay Performance with Variable Cell Number

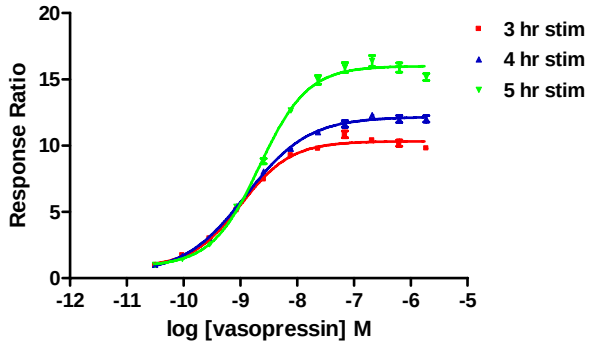
Figure 5— GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to Vasopressin using 1.25, 2.5, 5, and 10K cells / well



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells were plated at 1,250 2,500 or 5,000 and 10,000 cells/well in a 384-well format and incubated for 16-24 hours. On the day of the assay, cells were stimulated with Vasopressin (Phoenix Pharmaceuticals #065-10) 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 Vasopressin (n=8 for each data point).

Assay Performance with Variable Stimulation Time

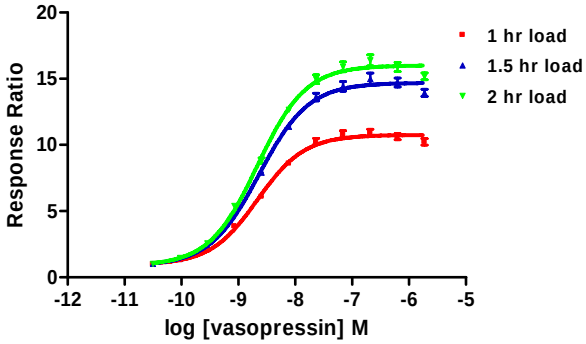
Figure 6 – GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to Vasopressin using 3, 4 and 5 hr stimulation times



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well assay plate and incubated for 16-24 hours. Vasopressin (Phoenix Pharmaceuticals #065-10) 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 against the indicated concentrations of Vasopressin (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

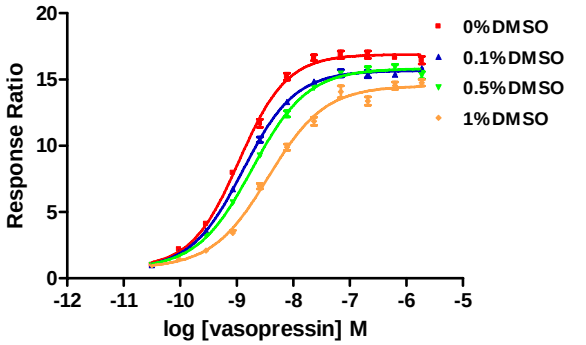
Figure 7— GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to Vasopressin using 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well black-walled tissue culture assay plate and incubated for 16-24 hours. Vasopressin (Phoenix Pharmaceuticals #065-10) was then added to the plate over the indicated concentration range in 0.5% DMSO for 5 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 Vasopressin (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® AVPR1a-NFAT-*bla* CHO-K1 dose response to Vasopressin using 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® AVPR1a NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well assay plate and incubated for 16-24 hours. Vasopressin (Phoenix Pharmaceuticals #065-10) 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 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 for each DMSO concentration against the indicated concentrations of Vasopressin (n=8 for each data point).

References

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