
Optimization of the GeneBLAzer® AVPR2 NFAT-bla CHO-K1 Cell Line

GeneBLAzer® AVPR2 CHO-K1 DA Assay Kit**GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 Cells**

Catalog Numbers – K1329 and K1718

Cell Line Descriptions

GeneBLAzer® AVPR2 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 cells contain the human Arginine Vasopressin Receptor 2 (AVPR2), (Accession # NM_0000504) 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 CRE 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® AVPR2 CHO-K1 DA cells and GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC₅₀ concentrations of [deamino-Cys1, D-Arg8]-Vasopressin acetate hydrate (dDAVP) (Figure 1). In addition, GeneBLAzer® AVPR2-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. [deamino-Cys1, D-Arg8]-Vasopressin acetate hydrate (dDAVP) dose response under optimized conditions

	DA cells	Dividing Cells
EC ₅₀	0.3 nM	0.3 nM
Z'-factor	0.92	0.92

Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 0 nM

2. Alternate agonist dose response

dDAVP EC ₅₀	= 129 pM
ARG-8 EC ₅₀	= 110 nM
V2013 EC ₅₀	= 1.35 nM

3. Antagonist dose response

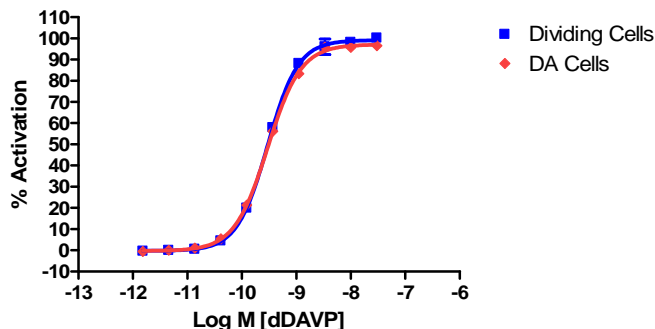
V4253 IC ₅₀	= 92.7nM
V2381 IC ₅₀	= 556 nM

Assay Testing Summary

- Assay performance in 2nd messenger assay.
- Assay performance with variable cell number.
- Assay performance with variable stimulation time.

Primary Agonist Dose Response

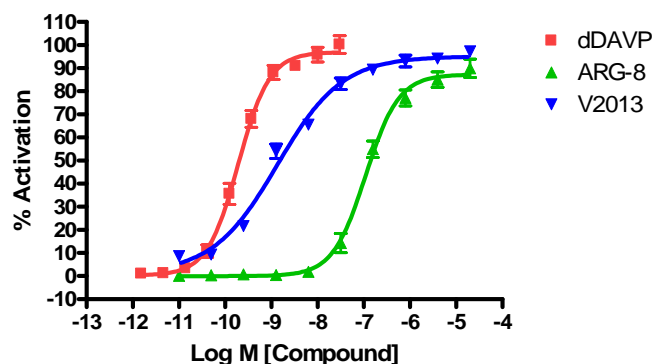
Figure 1 — GeneBLAzer® AVPR2 CHO-K1 DA and GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 cells dose response to dDAVP under optimized conditions



GeneBLAzer® AVPR2 CHO-K1 DA cells and GeneBLAzer® AVPR2-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 dDAVP (Sigma V1005) in the presence of 0.1% 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 [deamino-Cys1, D-Arg8]-Vasopressin acetate hydrate (dDAVP).

Alternate Agonist Dose Response

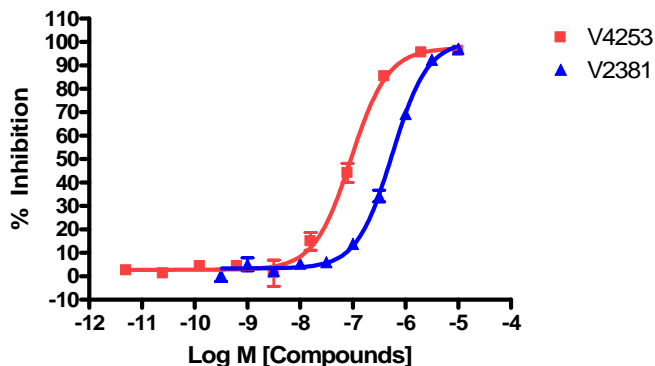
Figure 2 — GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 dose response to dDAVP, ARG-8 and V2013.



GeneBLAzer® AVPR2-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 dDAVP (Sigma V1005), ARG-8 (Sigma V2013) or V2013 (Sigma V9879) over the indicated concentration range in the presence of 0.1% DMSO for 5 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.

Antagonist Dose Response

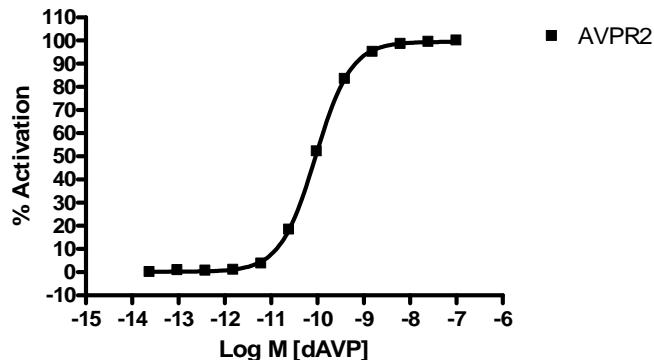
Figure 3 — GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 dose response to [β -Mercapto- β , β -cyclopentamethylenepropionyl1, O-Et-Tyr2, Val4, Arg8]-Vasopressin (V4253) and [Adamantaneacetyl1, O-Et-D-Tyr2, Val4, Aminobutyryl6, Arg8,9]-Vasopressin (V2381).



GeneBLAzer® AVPR2CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to [β -Mercapto- β , β -cyclopentamethylenepropionyl1, O-Et-Tyr2, Val4, Arg8]-Vasopressin (Sigma V4253) and [Adamantaneacetyl1, O-Et-D-Tyr2, Val4, Aminobutyryl6, Arg8,9]-Vasopressin (Sigma V2381) for 30 min. and then stimulated with an EC80 concentration of dDAVP (Sigma V1005) in the presence of 0.1% 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 substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of the antagonists.

2nd Messenger Dose Response

Figure 4 — GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 2nd messenger dose response to dDAVP under optimized conditions.



GeneBLAzer® AVPR2-CRE-*bla* CHO-K1 cells were tested for a response to dDAVP with a TR-FRET cAMP kit.