GeneBLAzer® ADRA1A CHO-K1 DA Cells

GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1577 and K1470

Cell Line Descriptions

GeneBLAzer® ADRA1A CHO-K1 DA(Division Arrested) cells and GeneBLAzer ® ADRA1A-NFAT-*bla* CHO-K1 cells contain the human Adrenergic Alpha-1A Receptor (ADRA1A) (ADRA1A), (Accession # NM_000680) stably integrated into the CellSensor® NFAT-bla CHO-K1 cell line. CellSensor® NFAT-bla CHO-K1 cells (Cat. no. K1534) contain a beta-lactamase reporter gene under control of the NFAT.

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® ADRA1A CHO-K1 DA cells and GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Phenylephrine (Figure 1). In addition, ADRA1A-NFAT-*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. Phenylephrine dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	23.7 nM	35.3 nM
Z'-factor	0.84	0.78

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

2. Alternate agonist dose response

Cirazoline EC_{50} = 1.2 nM
Clonidine EC_{50} = 10.3 nM
A61603 EC_{50} = 0.68 nM

3. Antagonist dose response

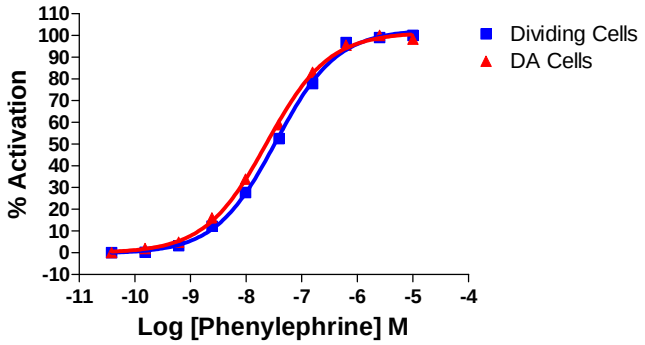
Prazosin:
Dividing IC_{50} = 0.9 nM
Cryopreserved IC_{50} = 1.2 nM
DA IC_{50} = 1.8 nM

4. Assay performance in 2nd messenger assay.

Phenylephrine EC_{50} = 10 nM
Clonidine EC_{50} = 23 nM

Primary Agonist Dose Response

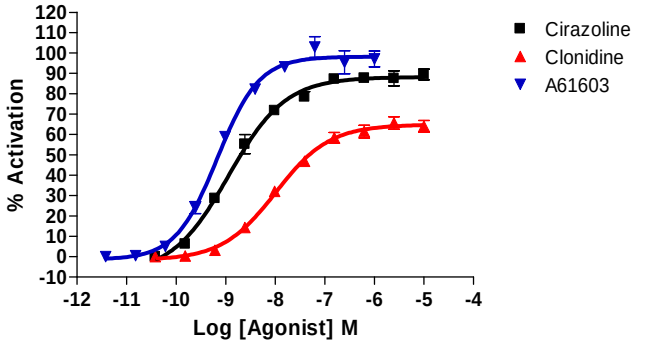
Figure 1 — GeneBLAzer® ADRA1A CHO-K1 DA and GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 cells dose response to Phenylephrine under optimized conditions



GeneBLAzer® ADRA1A CHO-K1 DA cells and GeneBLAzer® ADRA1A-NFAT-*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 Phenylephrine (Sigma P6126) 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 Phenylephrine.

Alternate Agonist Dose Response

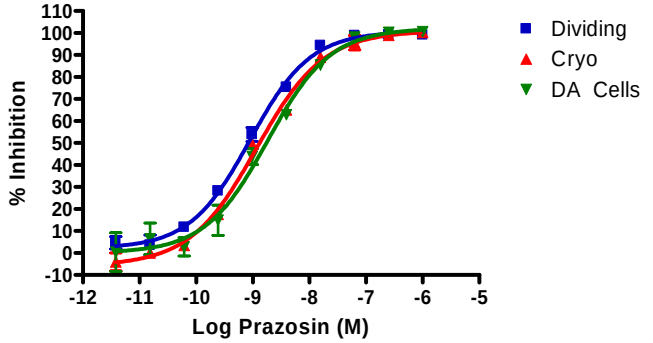
Figure 2 — GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 dose response to Cirazoline, Clonidine, and A61603.



GeneBLAzer® ADRA1A-NFAT-*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 Cirazoline (Tocris #0888), Clonidine (Tocris #0690), or A61603 (Tocris #1052) 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

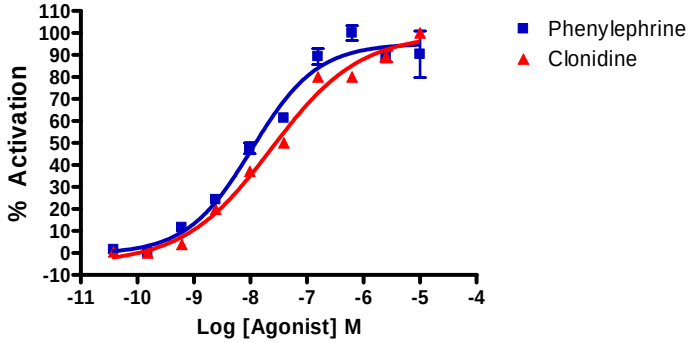
Figure 3 — GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 dose response to Prazosin



GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 cells or ADRA1A CHO-k1 DA cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Prazosin (Tocris 623) for 30 min. and then stimulated with an EC80 concentration of Phenylephrine (Sigma P6126) 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 Prazosin.

2nd Messenger Dose Response

Figure 4 — GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 2nd messenger dose response to Phenylephrine under optimized conditions.



GeneBLAzer® ADRA1A-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to Phenylephrine and Clonidine.