GeneBLAzer® ADRA1B CHO-K1 DA Assay Kit

GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1627 and K1471

Cell Line Descriptions

GeneBLAzer® ADRA1B CHO-K1 DA (Division Arrested) cells and GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 cells contain the human Adrenergic Alpha-1B Receptor (ADRA1B), (Accession # NM_000679.3) 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 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® ADRA1B CHO-K1 DA cells and GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Phenylephrine (Figure 1). In addition, GeneBLAzer® ADRA1B-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}	5.5 nM	12 nM
Z'-factor	0.78	0.74

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 10000 nM

2. Alternate agonist dose response

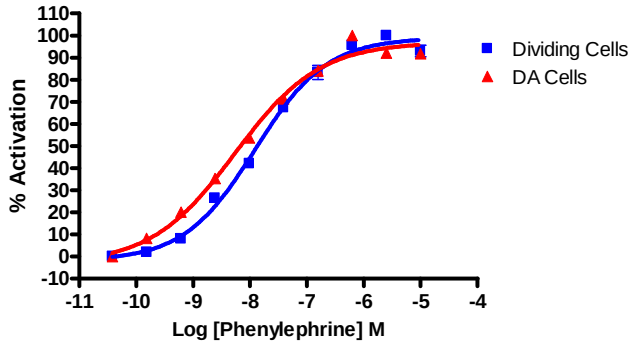
Cirazoline EC_{50} = 41.6 nM
Clonidine EC_{50} = 26.1 nM

3. Assay performance in 2nd messenger assay.

Phenylephrine EC_{50} = 2.1 nM

Primary Agonist Dose Response

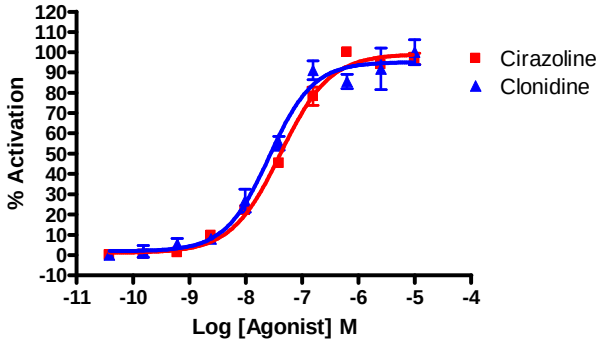
Figure 1 — GeneBLAzer® ADRA1B CHO-K1 DA and GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 cells dose response to Phenylephrine under optimized conditions



GeneBLAzer® ADRA1B CHO-K1 DA cells and GeneBLAzer® ADRA1B-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

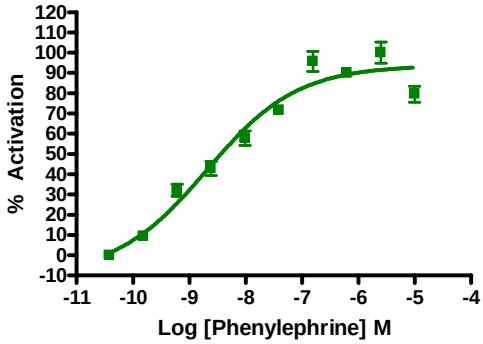
Figure 2 — GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 dose response to Cirazoline, Clonidine, and A61603.



GeneBLAzer® ADRA1B-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 dilution series of Cirazoline (Tocris #0888) or Clonidine (Tocris #0690) 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.

2nd Messenger Dose Response

Figure 3 — GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 2nd messenger dose response to Phenylephrine under optimized conditions.



GeneBLAzer® ADRA1B-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to Phenylephrine.