GeneBLAzer® ADRB1 CHO-K1 DA Cells

GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 Cells

Catalog Numbers -- K1589 and K1553

Cell Line Descriptions

GeneBLAzer® ADRB1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 cells contain the human Adrenergic Beta-1 Receptor (ADRB1), (Accession # NM_000684) stably integrated into the CellSensor® CRE-bla CHO-K1 cell line. CellSensor® CRE-bla CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase reporter gene under control of the CRE. 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 ®ADRB1 CHO-K1 DA cells and GeneBLAzer ® ADRB1-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of (-) Denopamine (Figure 1). In addition, GeneBLAzer ® ADRB1-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. (-) Denopamine dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	3.66 nM	4.07 nM
Z'-factor	0.83	0.83

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 20000 nM

2. Alternate agonist dose response

(-)-Epinephrine EC_{50} = 541.8 nM
(-)-Norepinephrine EC_{50} = 175.5 nM

3. Antagonist dose response

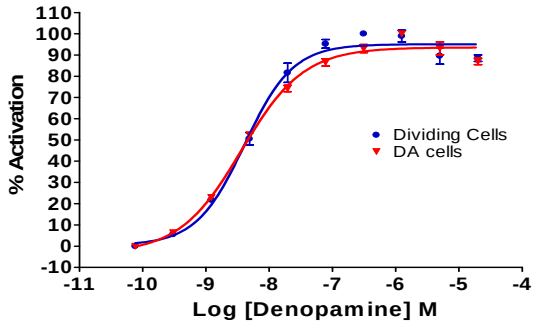
CGP20712A (Dividing) IC_{50} = 474.3 nM
CGP20712A (DA) IC_{50} = 356.4 nM

4. Agonist 2nd messenger dose response

Denopamine EC_{50} = 22 pM

Primary Agonist Dose Response

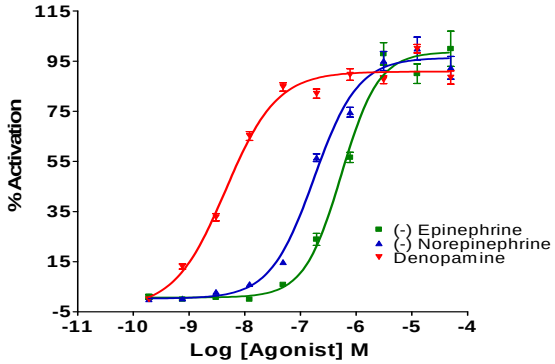
Figure 1 — GeneBLAzer® ADRB1 CHO-K1 DA and GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 cells dose response to (-) Denopamine under optimized conditions



GeneBLAzer® ADRB1 CHO-K1 DA cells and GeneBLAzer® ADRB1-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 (-) Denopamine (Sigma D7815) 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 plotted for each replicate against the concentrations of (-) Denopamine.

Alternate Agonist Dose Response

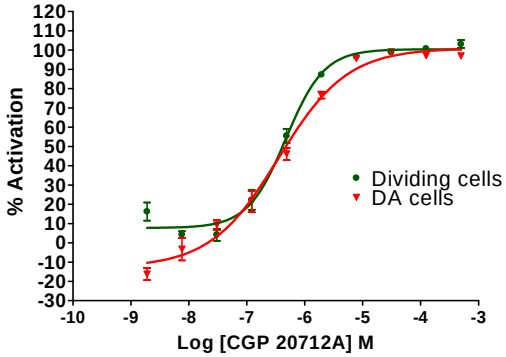
Figure 2 — GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 dose response to (-)-Epinephrine, (-)-Norepinephrine and Dopamine.



GeneBLAzer® ADRB1-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 (-)-Epinephrine (Sigma E4250), or (-)-Norepinephrine (Sigma A7257) or Denopamine (Sigma D7815) 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 460/530 Ratios plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

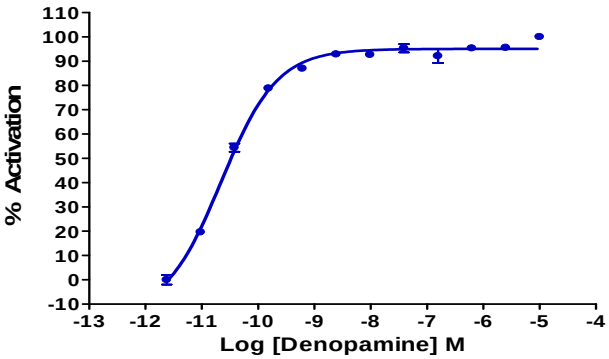
Figure 3 — GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 dose response to CGP 20712A



GeneBLAzer® GeneBLAzer® ADRB1-CRE-*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 CGP 20712A (Sigma C231) for 30 min. and then stimulated with an EC80 concentration of (-) Denopamine (Sigma D7815) 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 CGP 20712A.

2nd Messenger Dose Response

Figure 4 — GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 2nd messenger dose response to (-) Denopamine under optimized conditions.



GeneBLAzer® ADRB1-CRE-*bla* CHO-K1 cells were tested for a response to (-) Denopamine with a TR-FRET cAMP kit.