GeneBLAzer® D5 CHO-K1 DA Cells

GeneBLAzer® D5-CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1776 and K1784

Cell Line Descriptions

GeneBLAzer® D5 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® D5-CRE-*bla* CHO-K1 cells contain the human Dopamine Receptor D5 (D5), (Accession #NM_000676) 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 in 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® D5 CHO-K1 DA cells and GeneBLAzer® D5-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Fenoldopam (Figure 1). In addition, GeneBLAzer® D5-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. Fenoldopam dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	18.4 nM	18.2nM
Z'-factor	0.58	0.67

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] =2500 nM

2. Agonist 2nd messenger dose response

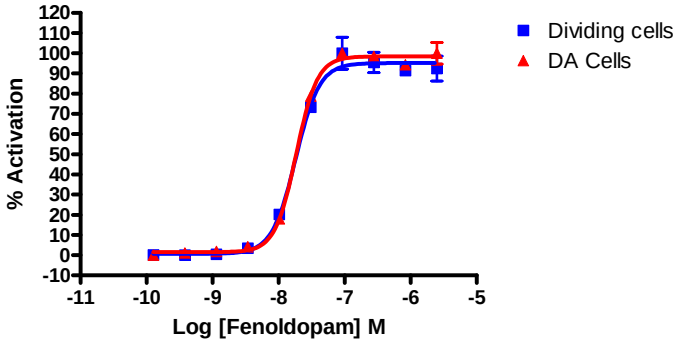
Fenoldopam EC_{50} = 409 pM

3. Antagonist dose response

Haloperidol IC_{50}= 93.6 nM dividing cells
58.1 nM DA cells

Primary Agonist Dose Response

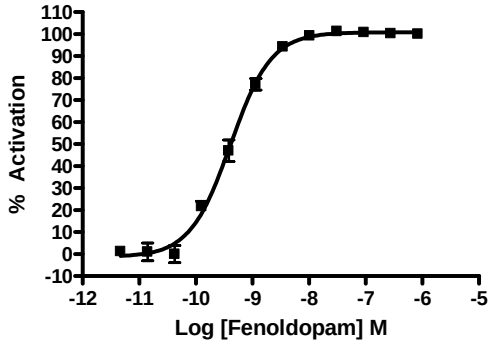
Figure 1 — GeneBLAzer® D5-CRE-*bla* CHO-K1 cells and D5-CRE-*bla* CHO-K1 DA cells dose response to Fenoldopam under optimized conditions



GeneBLAzer® D5 CHO-K1 DA cells and GeneBLAzer® D5-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 Fenoldopam (Sigma F6800) 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 Fenoldopam.

2nd Messenger Dose Response

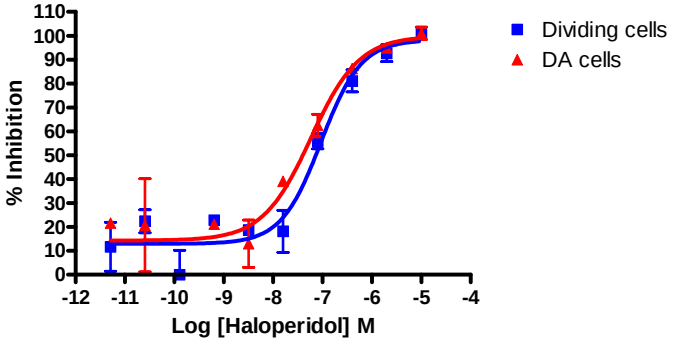
Figure 2 — GeneBLAzer® D5-CRE-*bla* CHO-K1 2nd messenger dose response to Fenoldopam under optimized conditions.



GeneBLAzer® D5-CRE-*bla* CHO-K1 cells were tested for a response to Fenoldopam (Sigma F6800) using a TR-FRET cAMP kit.

Antagonist Dose Response

Figure 3 — GeneBLAzer® D5-CRE-*bla* CHO-K1 and D5-CRE-*bla* CHO-K1 DA dose response to haloperidol



GeneBLAzer® D5-CRE-*bla* CHO-K1 cells and GeneBLAzer® D5-CRE-*bla* 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 haloperidol (Sigma H1512) for 30 min. and then stimulated with an EC80 concentration of Fenoldopam (Sigma F6800) 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 haloperidol.