GeneBLAzer® HCRTR1 CHO-K1 DA Cells

GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1580 and K1446

Cell Line Descriptions

GeneBLAzer® HCRTR1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 cells contain the human Hypocretin (Orexin) Receptor 1 (HCRTR1) (HCRTR1), (Accession # NM_001525) 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® HCRTR1 CHO-K1 DA cells and GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Orexin A (Figure 1). In addition, GeneBLAzer® HCRTR1-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. Orexin A dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1.63 nM	2.64 nM
Z'-factor	0.75	0.76

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 1000 nM

2. Alternate agonist dose response

Orexin B EC_{50} = 218.4 nM

3. Antagonist dose response

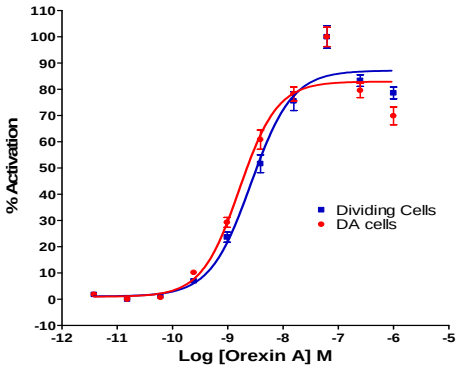
SB408124 (Dividing) IC_{50} = 2.0 µM
SB408124 (DA) IC_{50} = 2.5 µM

4. Agonist 2nd messenger dose response

Orexin A EC_{50} = 0.53 nM

Primary Agonist Dose Response

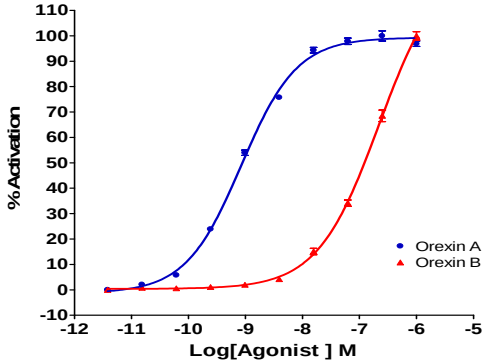
Figure 1 — GeneBLAzer® HCRTR1 CHO-K1 DA and GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 cells dose response to Orexin A under optimized conditions



GeneBLAzer® HCRTR1 CHO-K1 DA cells and GeneBLAzer® HCRTR1-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 Orexin A (Sigma O6012) 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 Orexin A.

Alternate Agonist Dose Response

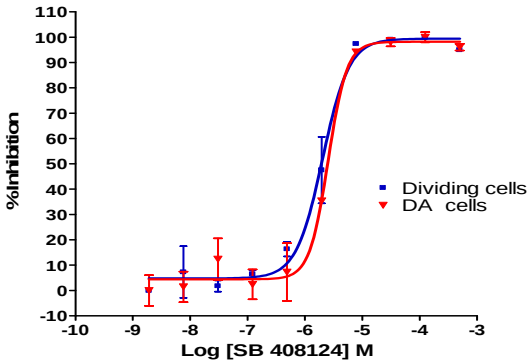
Figure 2 — GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 dose response to Orexin B and Orexin A.



GeneBLAzer® HCRTR1-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 Orexin B (Sigma O6137), or Orexin A (Sigma O6012) 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

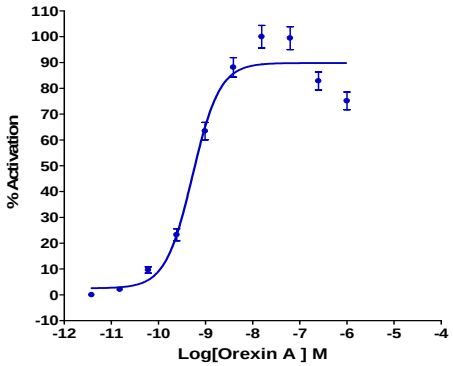
Figure 3 — GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 dose response to SB 408124



GeneBLAzer® HCRTR1-NFAT-*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 SB 408124 (Sigma S2694) for 30 min. and then stimulated with an EC80 concentration of Orexin A (Sigma O6012) 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 % Inhibition plotted against the indicated concentrations of SB 408124.

2nd Messenger Dose Response

Figure 4 — GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 2nd messenger dose response to Orexin A under optimized conditions.



GeneBLAzer® HCRTR1-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to Orexin A.