GeneBLAzer® GRPR CHO-K1 DA Cells

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

Catalog Numbers – K1637 and K1561

Cell Line Descriptions

GeneBLAzer® GRPR CHO-K1 DA (Division Arrested) cells and GeneBLAzer ® GRPR-NFAT-*bla* CHO-K1 cells contain the human Gastrin Releasing Peptide Receptor (GRPR), (Accession # NM_005314.2) 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 ® GRPR CHO-K1 DA cells and GeneBLAzer ® GRPR-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of GRP (Figure 1). In addition, GeneBLAzer ® GRPR-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. GRP dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	91 pM	52 pM
Z'-factor	0.76	0.53

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 100 nM

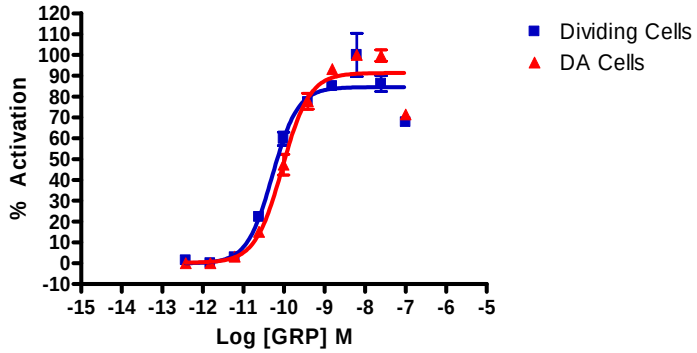
2. Antagonist dose response

ICI 216,140:
Dividing IC_{50} = 74 nM
Cryopreserved IC_{50} = 98.4 nM
DA IC_{50} = 80 nM

3. Assay performance with variable cell number.

Primary Agonist Dose Response

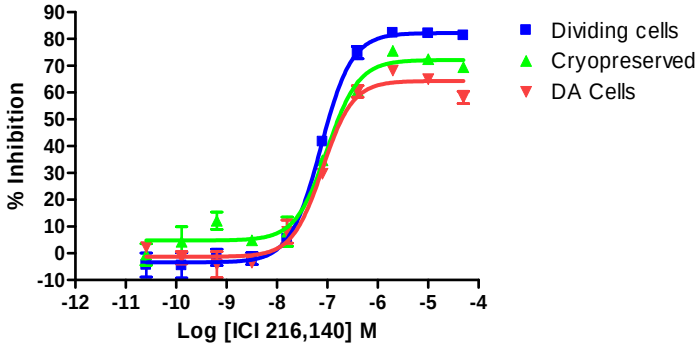
Figure 1 — GeneBLAzer® GRPR CHO-K1 DA and GeneBLAzer® GRPR-NFAT-*bla* CHO-K1 cells dose response to GRP under optimized conditions



GeneBLAzer® GRPR CHO-K1 DA cells and GeneBLAzer® GRPR-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 GRP (Tocris 1789) 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 GRP.

Antagonist Dose Response

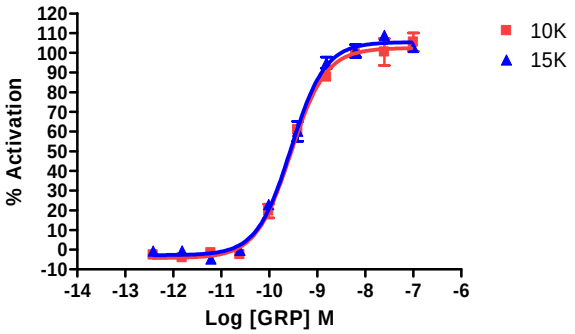
Figure 2 — GeneBLAzer® GRPR-NFAT-*bla* CHO-K1 dose response to ICI216,140



GeneBLAzer® GRPR-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 ICI216,140 (Tocris 823) for 30 min. and then stimulated with an EC80 concentration of GRP (Tocris 1789) 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 ICI216,140.

Assay Performance with Variable Cell Number

Figure 3 — GeneBLAzer® GRPR-NFAT-*bla* CHO-K1 cells dose response to GRPwith 10K or 15K cells/well



GeneBLAzer® GRPR-NFAT-*bla* CHO-K1 cells were plated in a 384-well format at 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with GRP (Tocris 1789) 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 cell numbers were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of GRP.