GeneBLAzer® CRHR1 CHO-K1 DA Assay Kit

GeneBLAzer® CRHR1 CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1367 and K1736

Cell Line Descriptions

GeneBLAzer® CRHR1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells contain the human Corticotropin Releasing Factor 1 (CRHR1) receptor (Accession # NM_004382.2) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase (*bla*) reporter gene under control of the Cyclic AMP Response Element (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® CRHR1 CHO-K1 DA cells and GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of corticotropin releasing factor (CRF), (Figure 1). In addition, GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also included.

Target Description

Corticotropin releasing factor (CRF) is a 41-amino acid peptide that plays a role in the integration of autonomic, neuroendocrine, and behavioral responses to stress. These effects are mediated through two receptor families, CRHR1 and CRHR2. While CRF was originally isolated from the hypothalamus, where it was shown to be the primary neuroregulator mediating the hypothalamic-pituitary-adrenocortical stress axis, it has since been found to be widely distributed outside the hypothalamus throughout the central nervous system.

Presently, there are five distinct targets for CRF with unique pharmacology and localization. These have been placed into three distinct classes, two of which are the G-protein coupled receptors CRF_1 (CRHR1) and CRF_2 (CRHR2). The CRHR1 receptor subtype is localized primarily to the cortical and cerebellar regions. The natural mammalian ligand r/hCRF has been shown to exhibit high affinity for the CRHR1 receptor subtype.

The number of potential therapeutic indications for CRF receptor antagonists is increasing substantially. The primary focus for CRHR1 receptor antagonists is in the area of anxiety and depression, with potential impacts on the treatment of neurodegeneration associated with stroke, the pain associated with various inflammatory responses, and potential utility in irritable bowel syndrome.

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **CRF agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	17 pM	15 pM
Z'-factor	0.77	0.77

Recommended cell no. = 10K cells/well
Recommended [DMSO] = 0.1-1%
Recommended Stim. Time = 4 hours
Max. [Stimulation] = 333 pM

2. **Agonist 2nd Messenger Response**

CRF EC_{50} = 85 pM

3. **Antagonist Dose Response**

Astressin IC_{50} = 835 pM

Assay Testing Summary

4. **Assay performance with variable cell number**

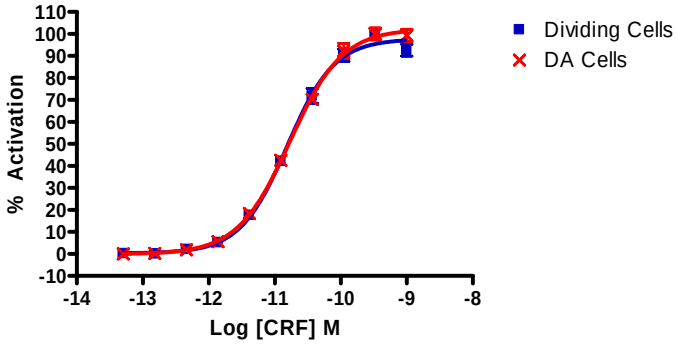
5. **Assay performance with variable stimulation time**

6. **Assay performance with variable substrate loading time**

7. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

Figure 1 — GeneBLAzer® CRHR1 CHO-K1 DA and CRHR1-CRE-*bla* CHO-K1 dose response to corticotropin releasing factor (CRF) under optimized conditions



GeneBLAzer® CRHR1 CHO-K1 DA cells and GeneBLAzer® CRHR1-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 corticotropin releasing factor (CRF) in the presence of 0.5% 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 CRF (n=6 for each data point).

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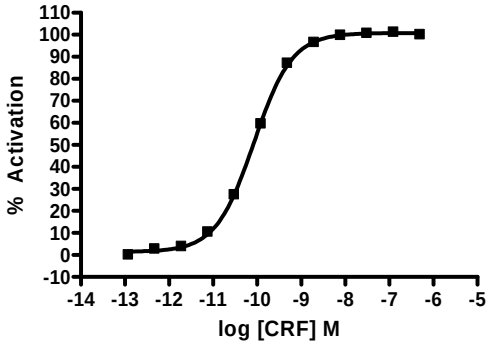
Agonist 2nd Messenger Response

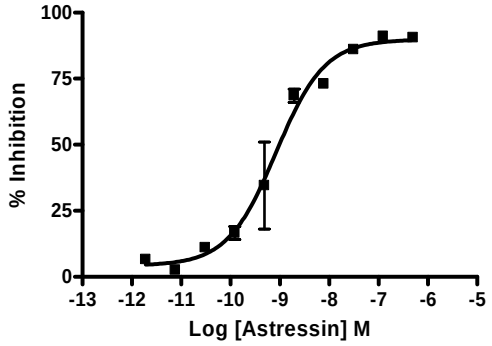
Figure 2— GeneBLAzer® CRHR1-CRE-*bla* CHO-k1 2nd messenger dose response to CRF under optimized conditions



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells were tested for a response to CRF with a TR-FRET cAMP assay.

Antagonist Dose Response

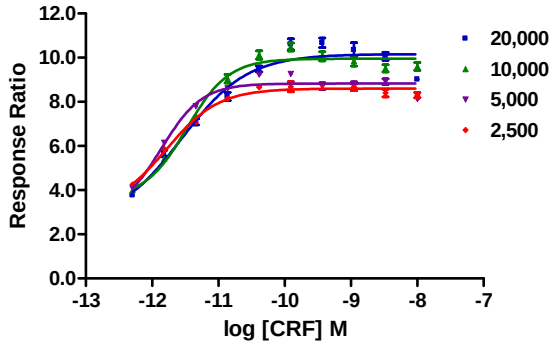
Figure 3— GeneBLAzer® CRHR1-CRE-*bla* CHO-k1 antagonist dose response to Astressin under optimized conditions



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well assay plate and incubated for 16-20 hours. Cells were then incubated with a dilution series of Astressin for 30 min. at 37°C followed by a 5 hour incubation with an EC_{80} concentration of CRF (Phoenix Pharmaceutical #019-06) in 0.1% DMSO. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of Astressin (n=2 for each data point).

Assay Performance with Variable Cell Number

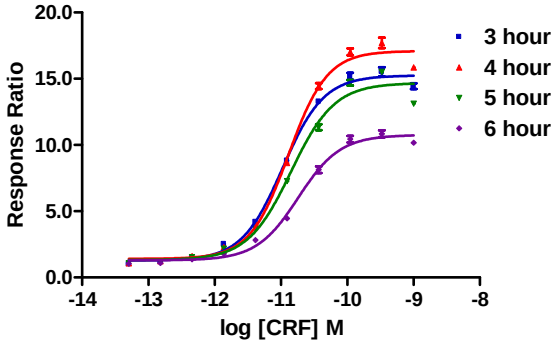
Figure 4 — GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 dose response to CRF with 2.5, 5, 10, and 20K cells/well



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells were plated the day before agonist addition at 2500, 5000, 10000, or 20,000 cells/well in a 384-well format. Cells were stimulated with CRF (Phoenix Pharmaceutical #019-06) in the presence of 0.5% 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 460/530 ratios plotted for each cell number against the indicated concentrations of CRF (n=8 for each data point).

Assay Performance with Variable Stimulation Time

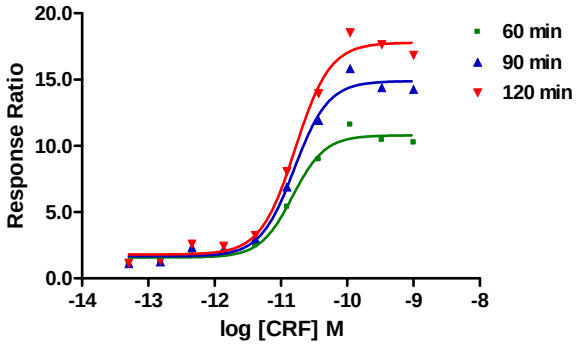
Figure 5 – GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 dose response to CRF with 3, 4, 5, and 6 hour stimulation times



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before agonist addition in a 384-well assay plate. CRF (Phoenix Pharmaceutical #019-06) was then added to the plate over the indicated concentration range. Plates were stimulated for 3, 4, 5 or 6 hrs with CRF in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each stimulation time against the indicated concentrations of CRF (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

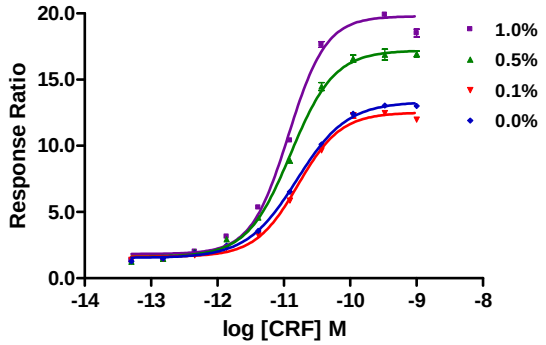
Figure 6 — GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 dose response to CRF with 60, 90 and 120 minute substrate loading times



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells were plated the day of the assay at 10,000 cells/well in a 384-well format. Cells were stimulated with CRF (Phoenix Pharmaceutical #019-06) over the indicated concentration range in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 60, 90, or 120 minutes. 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 Response Ratios plotted for each substrate loading time against the indicated concentrations of CRF (n=16 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 7 – GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 dose response to CRF with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® CRHR1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before agonist addition in a 384-well black-walled tissue culture assay plate. DMSO was then added to the assay at concentrations from 0% to 1%, and CRF (Phoenix Pharmaceutical #019-06) was added to the plate over the indicated concentration range. Plates were stimulated for 4 hrs and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios for each DMSO concentration were plotted against the indicated concentrations of CRF (n=8 for each data point).