GeneBLAzer® CCKBR HEK 293T DA Assay Kit

GeneBLAzer® CCKBR NFAT-*bla* HEK 293T Cells

Catalog Numbers – K1379 and K1742

Cell Line Descriptions

GeneBLAzer® CCKBR HEK 293T DA (Division Arrested) cells and GeneBLAzer® CCKBR-NFAT-*bla* HEK 293T cells contain the human Cholecystokinin (CCKBR) receptor (Accession # NM_176875) stably integrated into the CellSensor® NFAT-*bla* HEK 293T cell line. CellSensor® NFAT-*bla* HEK 293T cells (Cat. no. K1538) contain a beta-lactamase (*bla*) reporter gene under control of the nuclear factor of activated T-cells (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® CCKBR HEK 293T DA cells and GeneBLAzer® CCKBR-NFAT-*bla* HEK 293T cells are functionally validated for Z'-factor and EC_{50} concentrations of cholecystokinin (CCK). In addition, GeneBLAzer® CCKBR-NFAT-*bla* HEK 293T cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time.

Target Description

There are two cholecystokinin receptors, referred to as A and B (CCKAR and CCKBR). Both are Class A (rhodopsin-like) GPCRs, have been shown to signal through either Gs or Gq coupled pathways (1-4) and are 48% homologous. These two receptors differ in tissue distribution and agonist binding properties. CCKAR is detected in many tissues of the gastrointestinal system and a few areas of the brain, while CCKBR can be found in many areas of the brain and the stomach (5, 6). The receptors can be activated by different fragments of the cholecystokinin peptide. CCKAR requires a larger fragment, CCK-8, to be activated. CCKBR can be activated by the smaller CCK-4 (7, 8). In addition, CCKBR is also stimulated by gastrin. The stomach gastrin receptor and the CCKBR receptor in the brain were found to be the same protein (6).

CCK is widely distributed throughout the CNS and digestive system. Through CCKAR and CCKBR, it is thought to play a role in many digestive processes as well as anxiety, depression, psychosis, cognition and nociception (reviewed in 9). Studies have shown CCKBR could be involved in anxiety (10), learning and memory (11), as well as growth of gastric mucosa (12).

Validation Results

Performance of this assay was evaluated under various conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. CCK agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	0.6 nM	0.6 nM
Z'-factor	0.84	0.79

Optimum cell no.	= 5K cells/well
Optimum [DMSO]	= 0.25%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 100nM

2. Alternate agonist dose response

Pentagastrin EC_{50} = 1.1 nM

3. Antagonist dose response

None

4. Agonist 2nd Messenger Response

CCK EC_{50} = 6 nM

Assay Performance with Variable Conditions

5. Assay performance with variable cell number

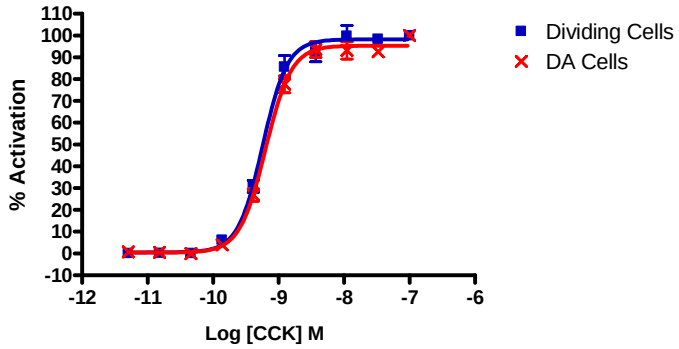
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

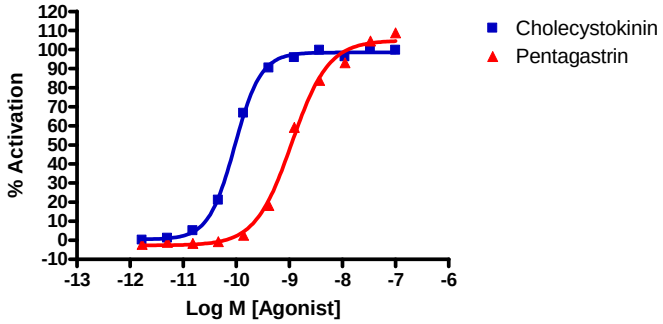
Figure 1 — GeneBLAzer® CCKBR HEK 293T DA and CCKBR-NFAT-*bla* HEK 293T dose response to CCK under optimized conditions.



GeneBLAzer® CCKBR HEK 293T DA cells and GeneBLAzer® CCKBR-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of CCK 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 plotted as % Activaton for each replicate against the concentrations of CCK (n=6 for each data point).

Alternate Agonists Dose Response

Figure 2 — GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 dose response to Pentagastrin and CCK



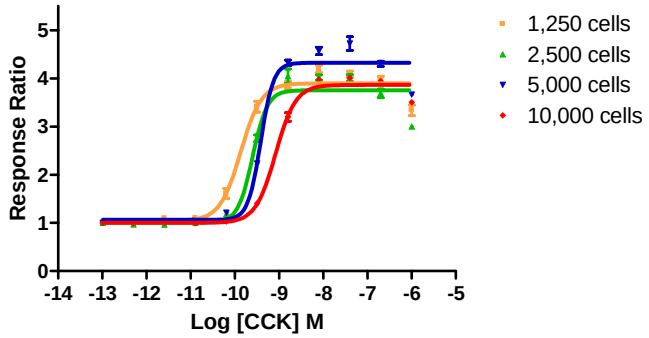
GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either CCK (Sigma #C2175) or pentagastrin (Sigma #B1636), over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded 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 the % Activation plotted against the indicated concentrations of agonist (n=16 for each data point). The data shows the correct rank order potency for these agonists.

Antagonist Dose Response

None

Assay Performance with Variable Cell Number

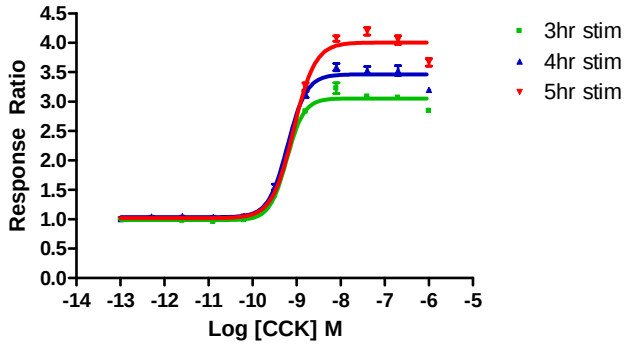
Figure 3— GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 dose response to CCK using 1.25, 2.5, 5, and 10K cells / well



GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 cells were plated the day before the assay at 1,250 2,500 or 5,000 and 10,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with CCK (Sigma #C2175) 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 Response Ratios plotted for each cell number against the indicated concentrations of CCK (n=8 for each data point).

Assay Performance with Variable Stimulation Time

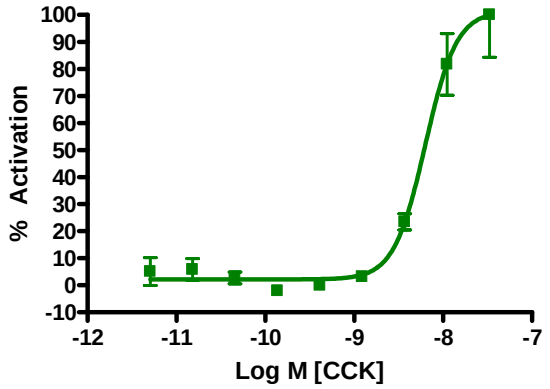
Figure 5 – GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 dose response to CCK with 3, 4 and 5 hr stimulation times



GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated the day before the assay in a 384-well assay plate. CCK (Sigma #C2175) was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs 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 against the indicated concentrations of CCK (n=8 for each data point).

Agonist 2nd Messenger Response

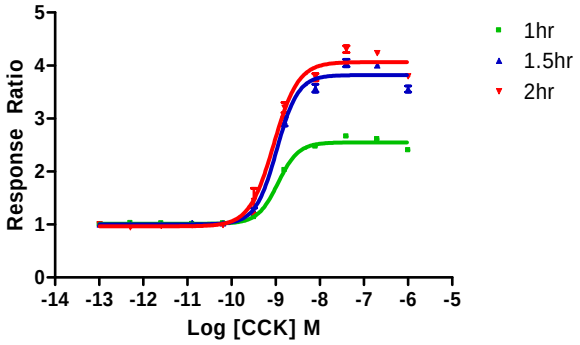
Figure 4— GeneBLAzer® CCKBR-NFAT-*bla* CHO-k1 2nd messenger dose response to CCK under optimized conditions



GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response CCK.

Assay Performance with Variable Substrate Loading Times

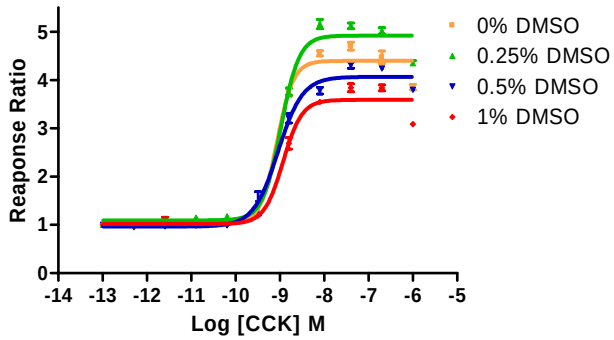
Figure 6— GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 dose response to CCK with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® CKBR NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. CCK (Sigma #C2175) was then added to the plate over the indicated concentration range in 0.5% DMSO for 3 hours and then loaded for 1, 1.5 or 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 substrate loading time plotted against the indicated concentrations of CCK (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 7 – GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 dose response to CCK with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® CCKBR NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated the day before the assay in a 384-well assay plate. CCK (Sigma #C2175) was then added to the plate over the indicated concentration range. DMSO was added to separate wells at concentrations from 0% to 1%. Cells were stimulated for 3 hrs with agonist 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 are shown for each DMSO concentration against the indicated concentrations of CCK (n=8 for each data point).

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