
Optimization of the GeneBLAzer® Bradykinin B1 NFAT-*bla* CHO-K1 Cell Line

GeneBLAzer® Bradykinin B1 CHO-K1 DA Assay Kit**GeneBLAzer® Bradykinin B1 NFAT-*bla* CHO-K1 Cells**

Catalog Numbers – K1363 and K1734

Cell Line Descriptions

GeneBLAzer® Bradykinin B1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells contain the human Bradykinin B1 receptor (Accession # [NM_000710](#)) 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 nuclear factor of activated T-cell (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® Bradykinin B1 CHO-K1 DA cells and GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC₅₀ concentrations using bradykinin. In addition, GeneBLAzer® Bradykinin B1-NFAT-*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 available.

Target Description

Kinins are a group of peptides that are produced throughout the body in response to infection, trauma, or inflammation. Bradykinin and its derivatives are formed when kininogens are cleaved by kallikreins (reviewed in 1). Bradykinin (BK) exerts its biological effects through the Bradykinin receptors, B1 and B2. These receptors are involved in the typical symptoms of inflammation such as increased vascular permeability, edema formation, and pain (2-7). Both receptors belong to the rhodopsin-like family (Family A) of G-protein coupled receptors and can signal through Gq coupling (8, 9).

B1 is rapidly upregulated by injury or inflammatory events and is not expressed under normal conditions. This receptor can be expressed in many areas of the body and is believed to play a role in vascular tone, inflammation, pain and some allergies (reviewed in 10). B1 has high affinity for certain derivatives of BK including des-Arg⁹-BK and Lys-des-Arg⁹-BK (2,3).

Validation Results

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

1. [Lys-des-Arg⁹]-Bradykinin dose response under optimized conditions

	<u>DA cells</u>	<u>Dividing Cells</u>
EC ₅₀	7.2 nM	9.3 nM
Z'-factor	0.82	0.76

Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= up to 0.5%
Recommended Stim. Time	= 5 hours
Max. [Stimulation]	= 10 μM

2. Alternate agonist dose response

Bradykinin (1-8) EC₅₀ = 1.2 μM

3. Antagonist dose response

[des-Arg¹⁰]-HOE 140 = 177 nM

4. Agonist 2nd messenger response

[Lys-des-Arg⁹]-Bradykinin = 1.9 nM

Assay Performance with Variable Conditions

5. Assay performance with variable cell number

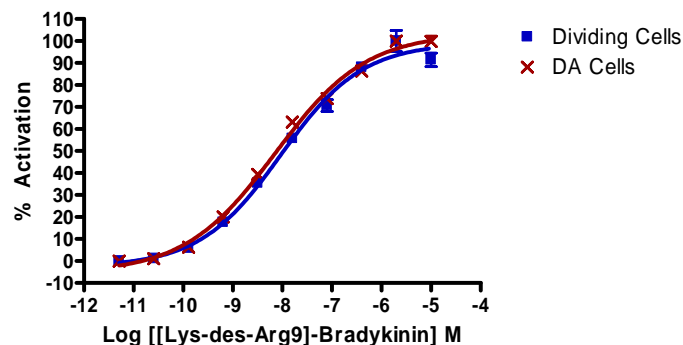
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

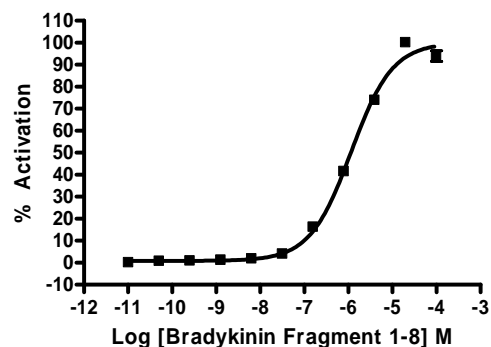
Figure 1 — GeneBLAzer® Bradykinin B1 CHO-K1 DA and GeneBLAzer® BradykininB1-NFAT-*bla* CHO-K1 dose response to [Lys-des-Arg⁹]-Bradykinin under optimized conditions.



GeneBLAzer® Bradykinin B1 CHO-K1 DA cells and BradykininB1-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 [Lys-des-Arg⁹]-Bradykinin 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 for each replicate against the concentrations of [Lys-des-Arg⁹]-Bradykinin (n=6 for each data point).

Alternate Agonist Dose Response

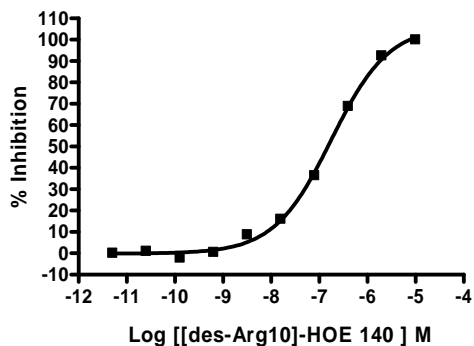
Figure 2 — GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response to Bradykinin (1-8)



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with Bradykinin fragment 1-8 (Sigma #B4397) 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 460/530 Emission Ratios are shown plotted against the indicated concentrations of agonist (n= 16 for each data point). The data shows the correct rank order potency relative to full length Bradykinin agonist.

Antagonist Dose Response

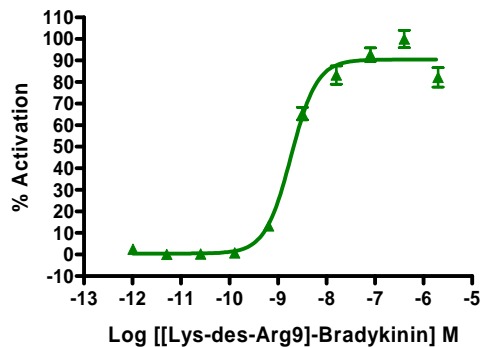
Figure 3 — GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response to [des-Arg¹⁰]-HOE 140 Antagonist



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well assay plate in. Cells were treated with [des-Arg¹⁰]-HOE 140 (Sigma Cat.#H-158) and incubated at 37 degrees C for 30 min., followed by 16nM [Lys-des-Arg⁹]-Bradykinin stimulation (Sigma Cat. #B1542) for 5 hours in 0.5% DMSO. Cells were 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 460/530 Emission Ratios are shown plotted against the indicated concentrations of the antagonist (n=8 for each data point).

Agonist 2nd Messenger Response

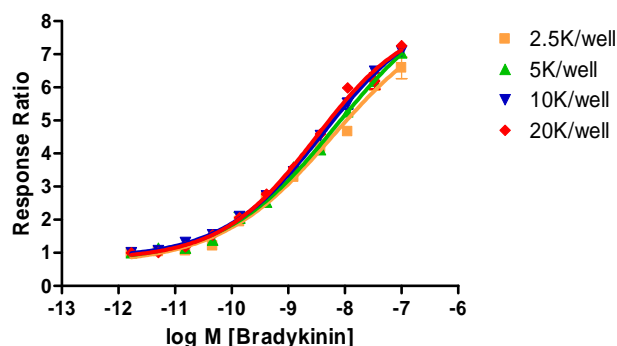
Figure 4— GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 2nd messenger dose response to [Lys-des-Arg⁸]-Bradykinin under optimized conditions



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to [Lys-des-Arg⁹]-Bradykinin].

Assay Performance with Variable Cell Number

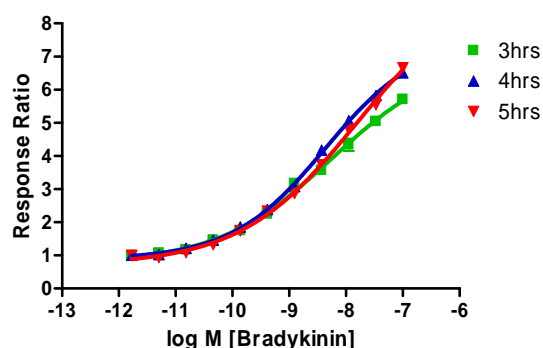
Figure 5— GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response with, 2.5, 5, 10 and 20K cells/well



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells were plated the day of the assay at 2500, 5000, 10000 or 20000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with Bradykinin (Sigma Cat. #B1542) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (2 μ M final concentration of CCF4-AM + 1 mM Solution D) 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 against the indicated concentrations of Bradykinin.

Assay Performance with Variable Stimulation Time

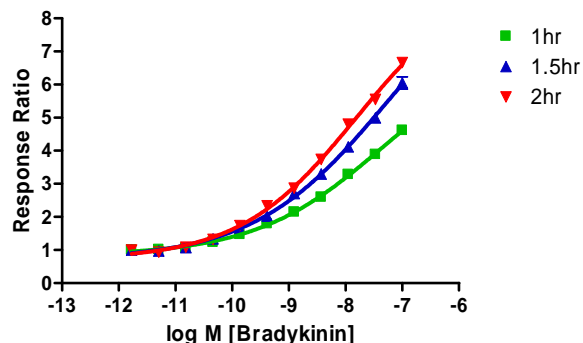
Figure 6 – GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response with 3, 4, and 5 hour stimulation times



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Bradykinin (Sigma Cat. #B1542) was then added to the plate over the indicated concentration range for 1, 2, 3, 4, or 5 hrs in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (2 μ M final concentration of CCF4-AM + 1 mM Solution D). 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 Bradykinin (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

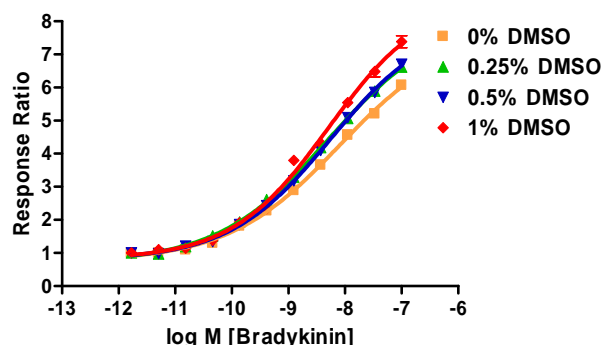
Figure 7 – GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response with 1, 1.5 and 2 hr substrate loading times



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells were plated the day of the assay at 20000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with Bradykinin (Sigma Cat. #B1542) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (2 μ M final concentration of CCF4-AM + 1 mM Solution D) for 1, 1.5 or 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 against the indicated concentrations of Bradykinin.

Assay Performance with variable DMSO concentration

Figure 8 – GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 dose response with 0, 0.25, 0.5 and 1% DMSO



GeneBLAzer® Bradykinin B1-NFAT-*bla* CHO-K1 cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Bradykinin (Sigma Cat. #B1542) was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 5 hrs with agonist and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (2 μ M final concentration of CCF4-AM + 1 mM Solution D). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios are shown plotted for each DMSO concentration against the indicated concentrations of Bradykinin (n=8 for each data point).

References

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