GeneBLAzer® ADRA2A-Gqo5 NFAT-*bla* CHO-K1 Cells

Catalog Numbers –K1743

Cell Line Descriptions

GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 cells contain the human Adrenergic Alpha 2A (ADRA2A) receptor (Accession # NM_000681) stably integrated into the GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cell line. GeneBLAzer® Gqo5-NFAT-*bla* CHO-K1 cells (Cat. no. K1536) contain the chimeric G-protein, Gqo5, in addition to a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells (NFAT) response element.

The GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of UK 14,304 (Figure 1). In addition, GeneBLAzer® ADRA2A-Gqo5-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 included.

Target Description

Adrenoceptors are a class of GPCRs that mediate the actions of the neurotransmitter noradrenalin and the hormone/neurotransmitter adrenaline. Adrenoceptors are separated into two major types, based on the rank order potencies of their agonists into α and β subtypes (1). The α subtype has been further broken down into 1α, 2α, and 3α subtypes based on molecular and pharmacological evidence (2). Three or four pharmacologically different subtypes of the Adrenoceptor 2α have been discovered. These subtypes were based on their affinity to prazosin (3). The A_{2A} receptor has a low affinity for the compound, where as the A_{2B} receptor's affinity is high. The A_{2C} receptor also has high affinity for prazosin but is different pharmacologically that the A_{2B} receptor. The fourth subtype A_{2D} is found in rat salivary gland and in the bovine pineal gland. This subtype is a species orthologue if A_{2A} and is not technically considered a subtype (4, 5).

Adrenoceptor A_{2A} (ADRA2A) is a major factor in the prejunctional control of neurotransmitter inhibition. It is also involved in vasoconstriction which makes this receptor an ideal target for hypertension medications. Other applications include treatment for Attention Deficit/Hyperactivity Disorder, glaucoma and anxiety (6).

ADRA2A receptors distributed widely throughout the body, including the brain, spleen and kidney. Other locations were ADRA2A receptors are found is the aorta, lung tissue, skeletal muscle, the heart and the liver (7, 8).

Validation Summary

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

1. UK 14,304 agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	23 nM	24 nM
Z'-factor	0.83	0.75

Recommended cell no. = 10K cells/well
Recommended [DMSO] = up to 1%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 10 µM

2. Alternate agonist dose response

(-)-Epinephrine EC_{50} = 95.7 nM
Guanabenz EC_{50} = 68.9 nM

3. Antagonist dose response

Yohimbine IC_{50} = 40 nM
BRL44408 IC_{50} = 218 nM

4. Agonist 2nd Messenger Response

UK14,304 EC_{50} = 19 nM

Assay Testing Summary

5. Assay performance with variable cell number

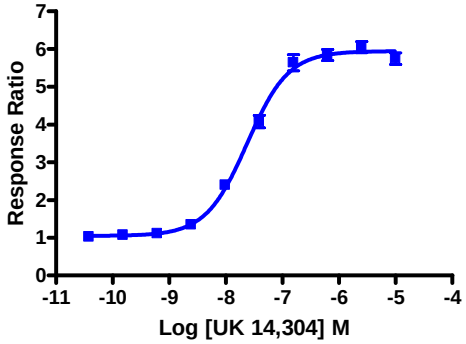
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

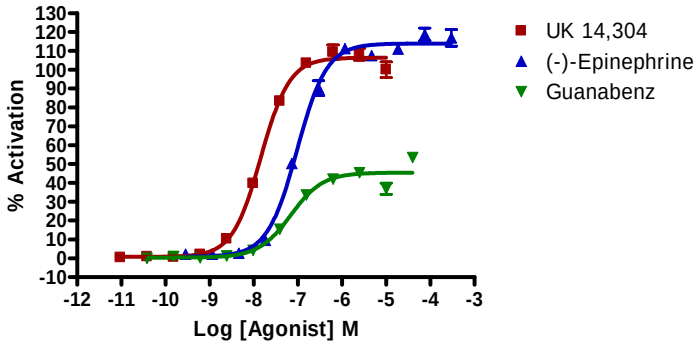
Figure 1 — GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304 under optimized conditions



GeneBLAzer® ADRA2A-GQO5-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 UK 14,304 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 was plotted for each replicate against the concentrations of UK 14,304 (n=6 for each data point).

Alternate Agonist Dose Response

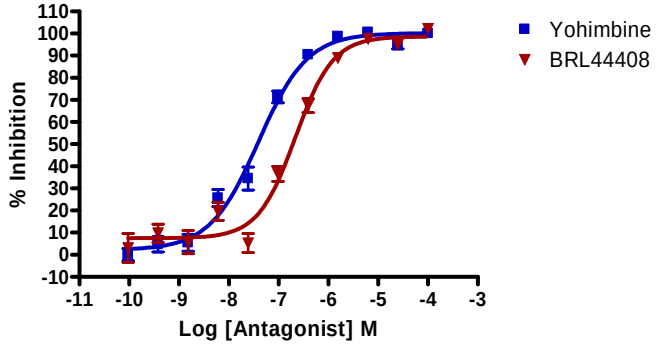
Figure 2 — GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304, (-)-Epinephrine and Guanabenz



GeneBLAzer® ADRA2A-Gqo5-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 dilution series of UK 14,304 (Sigma U104), Guanabenz (Sigma G110) and (-)-Epinephrine (Sigma E4250) 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 florescence plate reader and the % Activation is shown plotted against the concentrations of UK 14,304 and Adenosine (n=8 for each data point). The cell lines show the correct rank order potency for these compounds. It also shows the partial agonism of Guanabenz.

Antagonist Dose Response

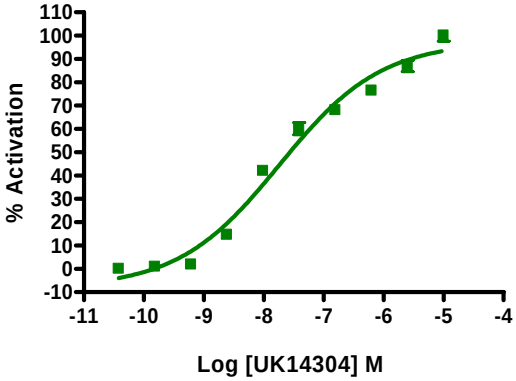
Figure 3 — GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to Yohimbine and BRL 44408



GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 cells were plated 16-20 hours prior to assay at 10,000 cells per well in a 384-well format. Cells were incubated at 37ºC & 5% CO_2 for 30 min. with a dilution series of BRL 44408 (Sigma #B4559) or Yohimbine (Sigma #Y3125) in the presence of 0.25% DMSO. UK 14,304 (Sigma #U104) was added to the plate at the EC_{80} concentration of 47.7 nM along with 0.25% DMSO (0.5% Final concentration). Cells were incubated for 5 hours 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 % Inhibition is shown plotted against the concentrations of the antagonists. This data shows the proper functioning of the assay in antagonist mode. (n=16 for each data point).

Agonist 2nd Messenger Response

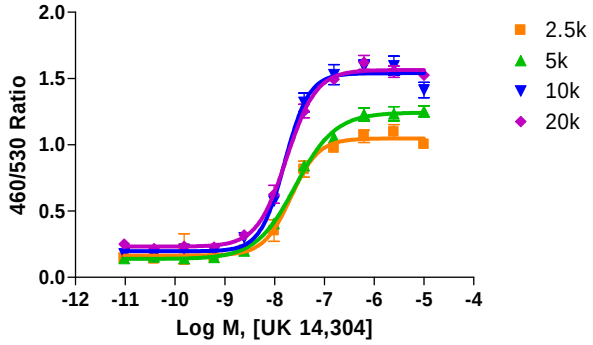
Figure 4— GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-k1 2nd messenger dose response to UK14304 under optimized conditions



GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to UK14304.

Assay Performance with Variable Cell Number

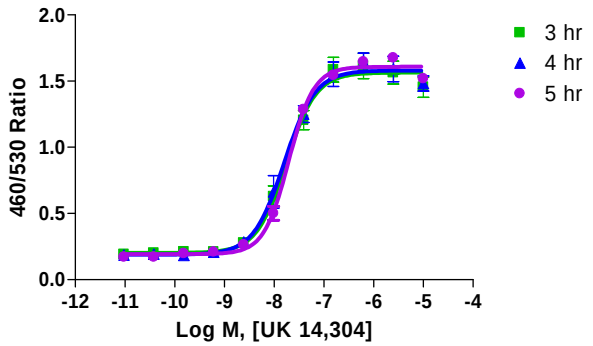
Figure 5 – GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304 with 2.5, 5, 10, and 20K cells/well



GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of UK 14,304 (Sigma U104) 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 and the Response Ratios plotted for each cell number against the concentrations of UK 14,304 (n=8 for each data point).

Assay Performance with Variable Stimulation Time

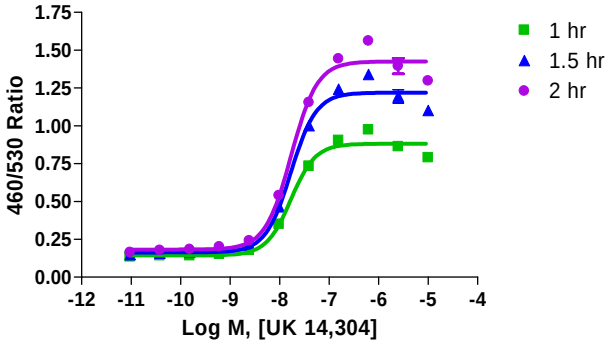
Figure 6 – GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304 with 3, 4 and 5 hr stimulation times



GeneBLAzer® ADRA2A-Gqo5-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 UK 14,304 (Sigma U104) for 3, 4, or 5 hrs in the presence of 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 florescence plate reader and the Response Ratios plotted for each stimulation time against the concentrations of UK 14,304 (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

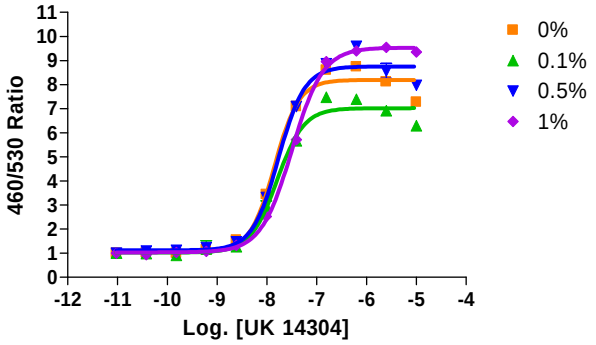
Figure 7 – GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304 with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® ADRA2A-Gqo5-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 UK 14,304 (Sigma U104) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 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 florescence plate reader and the Response Ratios plotted for each substrate loading time against the concentrations of UK 14,304 (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® ADRA2A-Gqo5-NFAT-*bla* CHO-K1 dose response to UK 14,304 with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® ADRA2A-Gqo5-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 UK 14,304 (Sigma U104) for 5 hours. DMSO was added to the cells at concentrations from 0% to 1%. 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 florescence plate reader and the Response Ratios plotted for each DMSO concentration against the concentrations of UK 14,304 (n=8 for each data point).

References

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