GeneBLAzer® ADRB3 CHO-K1 DA Cells

GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1473 and K1745

Cell Line Descriptions

GeneBLAzer® ADRB3 CHO-K1 DA (Division Arrested) cells and GeneBLAzer®ADRB3-CRE-*bla* CHO-K1 cells contain the human Adrenrgic Beta 3 Receptor (ADRB3) (Accession # NM_000025.1) 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).

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® ADRB3 CHO-K1 DA cells and GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Isoproterenol, (Figure 1). In addition, GeneBLAzer® ADRB3-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

Adrenoceptors are a class of GPCRs that mediate the actions of the neurotransmitter noradrenalin and the hormone/neurotransmitter adrenaline. The adrenoceptors were separated into two major types, based on the rank order potencies of their agonist's, 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). Initially the β-adrenoceptors were divided into two subtypes. The β1 receptor is dominant in the heart and adipose tissue and is equally activated by noradrenaline and adrenaline. The
B2 receptor is responsible for relaxation of uterine, vascular and airway smooth muscle and is less sensitive to noradrenaline than adrenaline (3). The β3 receptor is atypical as it is insensitive to common β-antagonists (4, 5).

Adrenoceptor B_3 (ADRB3) is a major factor in lipolysis and thermogenesis (6). These receptors are also involved in relaxation of the colon (7, 8). ADRB3 targeted drugs may have a negative inotropic effect (9), meaning that these drugs affect the force in which the heart contracts.

ADRB3 receptors distributed widely throughout the body, however they are found most frequently in adipose tissue and the gall bladder. Other locations were ADRB3 receptors are found is the small intestine, stomach, prostate, left atrium and bladder (10, 11).

Validation Summary

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

1. Isoproterenol dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	5.5 nM	4 nM
Z'-factor	0.75	0.66

Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= up to 1%
Recommended Stim. Time	= 5 hours
Max. [Stimulation]	= 600 nM

2. Alternate agonist dose response

(-)-Epinephrine EC_{50}	= 0.84 nM
BRL 37344 EC_{50}	= 0.74 nM

3. Antagonist dose response

ICI118551 IC_{50}	= 19 μM

4. Agonist 2nd Messenger Dose Response

Isoproterenol EC_{50}	= 1.1 nM

Assay Testing Summary

5. Assay performance with variable cell number

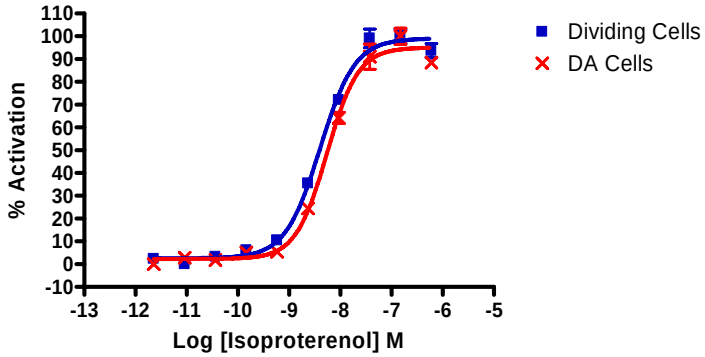
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

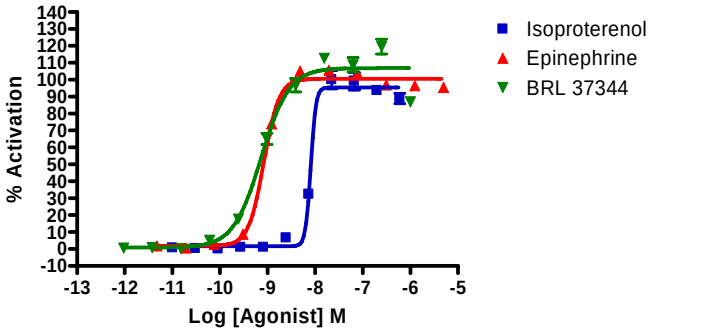
Figure 1 — GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 and GeneBLAzer® ADRB3 CHO-K1 DA Isoproterenol dose response under optimized conditions



GeneBLAzer® ADRB3 CHO-K1 DA and GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 cells (10,000 cells/well) were were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of Isoproterenol (Sigma #I5627) 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 Isoproterenol (n=16 for each data point).

Alternate Agonist Dose Response

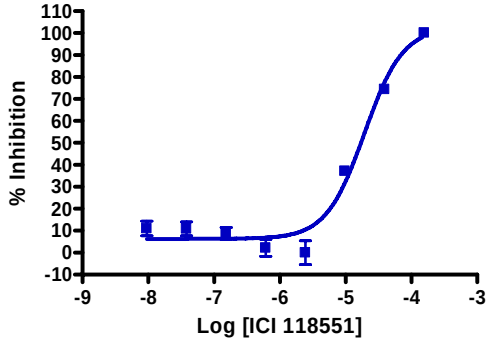
Figure 2 — GeneBLAzer® Isoproterenol, (-)-Epinephrine and BRL 37344 Dose Response



GeneBLAzer® ADRB3-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 dilution series of Isoproterenol (Sigma #I5627), BRL 37344 (Sigma #B169) 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 shown plotted against the concentrations of Isoproterenol, BRL 37344, and (-) Epinephrine (n=8 for each data point). The cell lines show the correct rank order potency for these compounds.

Antagonist Dose Response

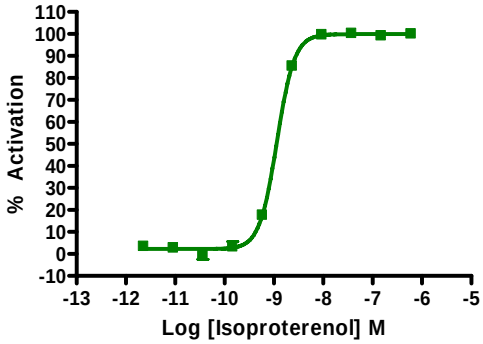
Figure 3 — GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 ICI 118,551 dose response



GeneBLAzer® ADRB3-CRE-*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 treated with a dilution series of ICI 118,551 (Sigma #I127) in the presence of 0.25% DMSO. Cells were then incubated at 37ºC & 5% CO_2 for 30 min. Isoproterenol (Sigma #I5627) was then added to the plate at the EC_{80} concentration of 26 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 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 Dose Response

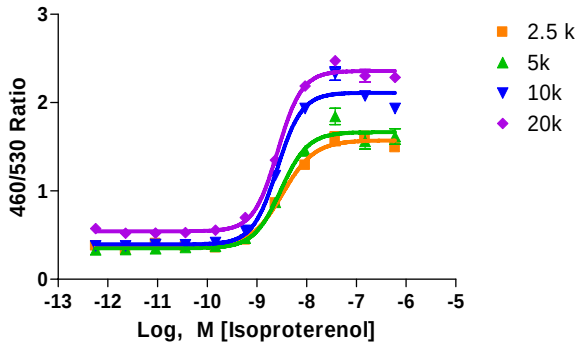
Figure 4— GeneBLAzer® ADRB3-CRE-*bla* CHO-k1 2nd messenger dose response to Isoproterenol under optimized conditions.



GeneBLAzer® ADRB3-CRE-*bla* CHO-K1 cells were tested for a response to isoproterenol with a TR-FRET cAMP assay

Assay Performance with Variable Cell Number

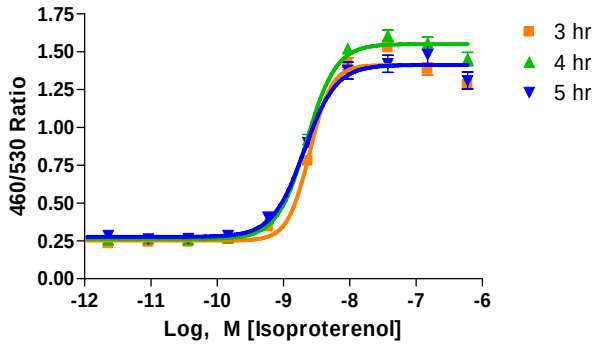
Figure 4 – Isoproterenol dose response with 2.5, 5, 10, and 20K cells/well



GeneBLAzer® ADRB3-CRE-*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 Isoproterenol (Sigma #I5627) 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 plotted for each cell number against the concentrations of Isoproterenol (n=8 for each data point).

Assay Performance with Variable Stimulation Time

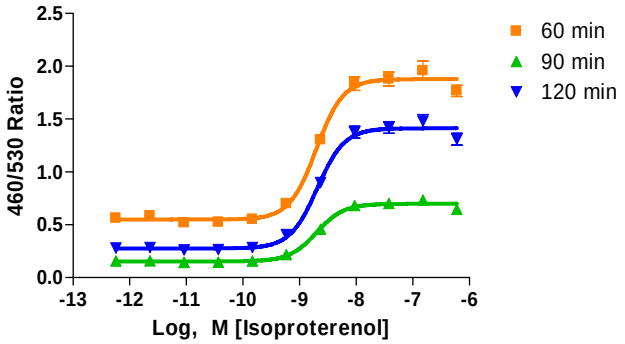
Figure 5 – Isoproterenol dose response with 3, 4 and 5 hr stimulation times



GeneBLAzer® ADRB3-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 Isoproterenol (Sigma #I5627) 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 plotted for each stimulation time against the concentrations of Isoproterenol (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

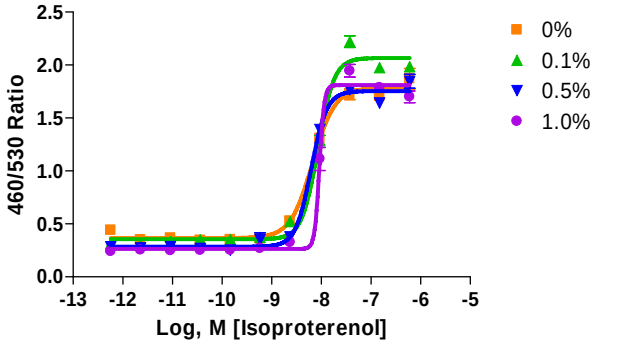
Figure 6 – GeneBLAzer® Isoproterenol dose response with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® ADRB3-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 Isoproterenol (Sigma #I5627) 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 fluorescence plate reader and plotted for each substrate loading time against the concentrations of Isoproterenol (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 7 – GeneBLAzer® Isoproterenol dose response with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® ADRB3-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 Isoproterenol (Sigma I5627) 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 fluorescence plate reader and plotted for each DMSO concentration against the concentrations of Isoproterenol (n=8 for each data point).

References

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