GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1472

Cell Line Descriptions

GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 cells contain the human Adrenergic Beta-2 Receptor (ADRB2), (Accession # NM_000024.3) stably integrated into the CellSensor® CRE-bla CHO-K1 cell line. CellSensor® CRE-bla CHO-K1 cells (Cat. no.K1535) contain a beta-lactamase reporter gene under control of the CRE. Division Arrested (DA) cells are available in two configurations: an Assay Kit (which includes cells and sufficient substrate to analyze 1 x 384-well plate), and a tube of cells sufficient to analyze 10 x 384-well plates.

The GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Isoproterenol (Figure 1). In addition, GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

Target Description

Adrenoceptor are a class 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).

The ADRB2 receptors are located on the lungs, kidneys, heart, brain skeletal muscle and liver tissues. These receptors are involved in bronchodilation, decrease in blood pressure, and uterine tissue relaxation.

The endogenous ligand for the ADRB2 receptor is epinephrine. Most antagonists to the ADRB2 receptor are bronchodilators. The agonists Procaterol, Fenoterol and Salbutamol are more commonly used in rescue inhalers for asthmatics. Salbutamol will also delay labor as it relaxes uterine tissues. Fenoterol has been removed from the market as there have been deaths associated with its use.

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}	5.3 nM	11.9 nM
Z'-factor	0.82	0.61

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 500 nM

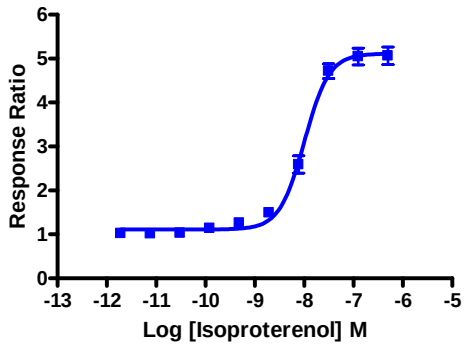
2. **Antagonist dose response**

Alprenolol hydrochloride IC_{50} = 6.33 nM

3. **Assay performance in 2nd messenger assay.**

Primary Agonist Dose Response

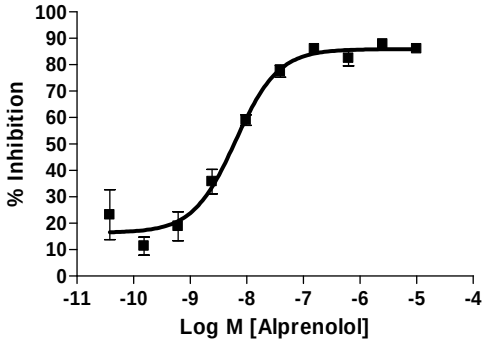
Figure 1 — GeneBLAzer® ADRB2 CHO-K1 DA and ADRB2-CRE-*bla* CHO-K1 cells dose response to Isoproterenol under optimized conditions



GeneBLAzer® ADRB2-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 0.1% 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.

Antagonist Dose Response

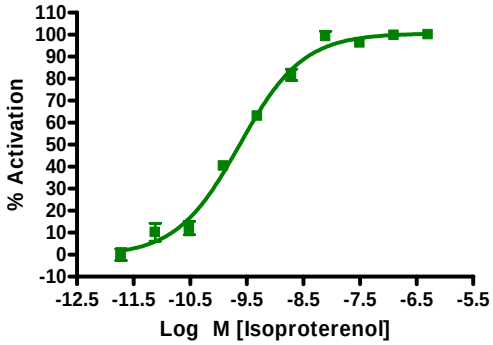
Figure 2 — GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 dose response to Alprenolol hydrochloride



GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Alprenolol hydrochloride (Sigma A8676) for 30 min. and then stimulated with an EC80 concentration of Isoproterenol (Sigma I5627) in the presence of 0.1% 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 substrate loading times were obtained using a standard fluorescence plate reader and the 460/530 Ratios plotted against the indicated concentrations of Alprenolol hydrochloride.

2nd Messenger Dose Response

Figure 3 — GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 2nd messenger dose response to Isoproterenol under optimized conditions.



GeneBLAzer® ADRB2-CRE-*bla* CHO-K1 cells were tested for a response to Isoproterenol with a TR-FRET cAMP kit.

References

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