

**GeneBLAzer® TREx-G2A-NFAT-*bla* Freestyle HEK 293F Cells**

Catalog Numbers – K1749

**Cell Line Descriptions**

GeneBLAzer® TREx™ G2A-NFAT-*bla* Freestyle HEK 293F cells contain the human GPR132 (G2A), (Accession # NP\_037477) stably integrated into the CellSensor® NFAT-*bla* Freestyle HEK 293F cell line. CellSensor® NFAT-*bla* Freestyle HEK 293F cells (Cat. no. K1725) contain a beta-lactamase reporter gene under control of the NFAT Response Element.

In addition, GeneBLAzer® TREx™ G2A-NFAT-*bla* Freestyle HEK 293F cells have been tested for assay performance under variable conditions and are functionally validated for Z'-factor and EC<sub>50</sub> concentrations of doxycycline (Figure 1).

## Validation Summary

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

### 1. Doxycycline dose response under optimized conditions

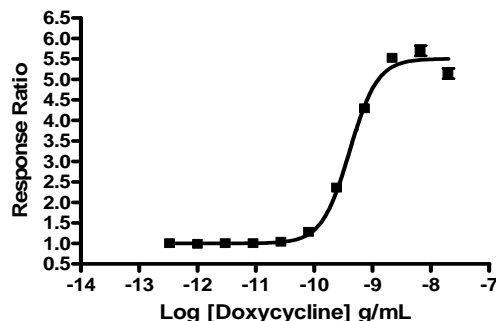
	<u>Dividing Cells</u>
EC <sub>50</sub>	= 0.131 ng/mL
Z'-factor	= 0.62
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 20 ng/mL

## Assay Testing Summary

### 2. Assay performance with variable cell number.

## Primary Agonist Dose Response

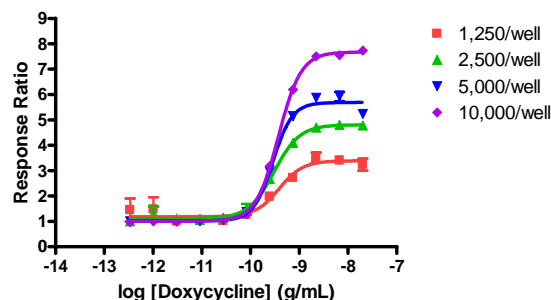
**Figure 1** — GeneBLAzer® TReX™ G2A-NFAT-*bla* Freestyle HEK 293F cells dose response to doxycycline under optimized conditions



GeneBLAzer® TReX™ G2A-NFAT-*bla* Freestyle HEK 293F 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 doxycycline (MP Biomedicals Cat #2195044.2) in the presence of 0.1% DMSO for 16 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 doxycycline.

## Assay Performance with Variable Cell Number

**Figure 2** — GeneBLAzer® TReX™ G2A-NFAT-*bla* Freestyle HEK 293F cells dose response to doxycycline with 1.25K, 2.5K, 5K, or 10K cells/well



GeneBLAzer® TReX™ G2A-NFAT-*bla* Freestyle HEK 293F cells were plated in a 384-well format at 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with doxycycline (MP Biomedicals Cat #2195044.2) in the presence of 0.1% DMSO for 16 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 RR plotted for each replicate against the concentrations of doxycycline.