GeneBLAzer® T-REx™ GPR119 CHO-K1 DA Assay Kit

GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1631 and K1486

Cell Line Descriptions

GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 DA(Division Arrested) cells and GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 cells contain the human G-Protein Receptor 119 (GPR119), (Accession # NP_848566) and stably integrated into the CRE-*bla* CHO-K1 cell line. CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase reporter gene under control of the cAMP response element.

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® T-REx™ GPR119 CHO-K1 DA cells and GeneBLAzer® T-REx GPR119-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of AR231453 (Figure 1). In addition, GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

Validation Summary

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

1. **AR231453 dose response under optimized conditions**

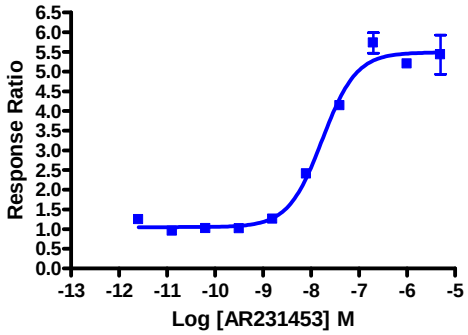
	DA cells	Dividing Cells
EC_{50}	25.2 nM	17.2 nM
Z'-factor	0.93	0.81

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 1000 nM

2. **Assay performance with or without receptor induction.**
3. **Assay performance with variable cell number.**
4. **Assay performance with variable stimulation time.**

Primary Agonist Dose Response

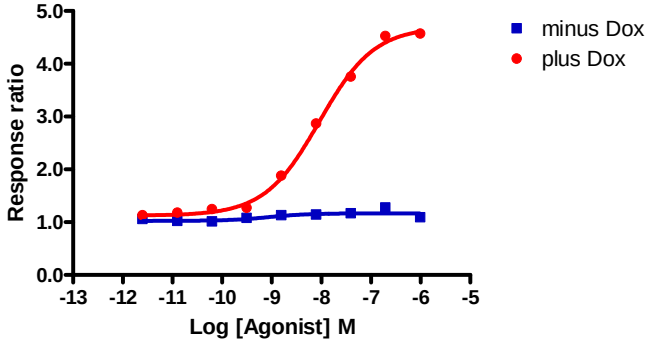
Figure 1 — GeneBLAzer® T-REx™ GPR119 CHO-k1 DA and T-REx GPR119-CRE-*bla* CHO-k1 cells dose response to AR231453 under optimized conditions



GeneBLAzer® T-REx GPR119 CHO-K1 DA cells and GeneBLAzer® T-REx GPR119-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 AR231453 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 plotted for each replicate against the concentrations of AR231453.

Inducible Agonist Dose Response

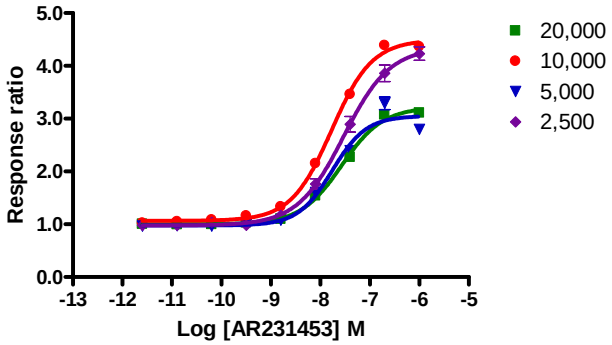
Figure 2 — GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 cells dose response to AR231453 with or without receptor induction.



GeneBLAzer® T-REx GPR119-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours in the presence or absence of 1 µg/mL doxycycline. Cells were stimulated with a dilution series of AR231453 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 plotted for each replicate against the concentrations of AR231453.

Assay Performance with Variable Cell Number

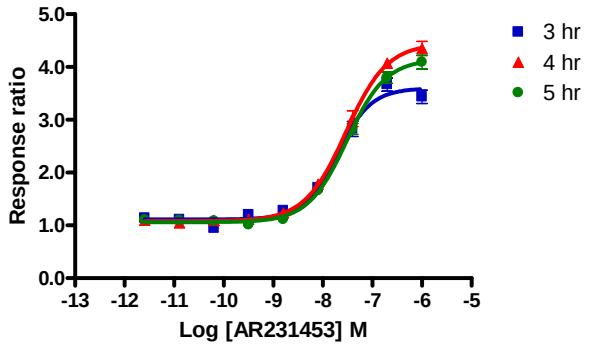
Figure 3 — GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 cells dose response to AR231453 with variable cells / well



GeneBLAzer® T-REx GPR119-CRE-*bla* CHO-K1 cells were plated in a 384-well format at 20,000; 10,000; 5,000; or 2,500 cells/well and incubated for 16 hours. On the day of the assay, cells were stimulated with AR231453 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 cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of AR231453.

Assay Performance with Variable Stimulation Time

Figure 4 – GeneBLAzer® T-REx™ GPR119-CRE-*bla* CHO-K1 cells dose response to AR231453 with 3, 4 and 5 hour stimulation times



GeneBLAzer® T-REx GPR119-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. AR231453 was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs in 0.1% DMSO. The 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 Response Ratios plotted against the indicated concentrations of AR231453.

References

1) Chu, Z-L, Jones, R. M., He, H., Carroll, C., Gutierrez, V., Lucman, A., Moloney, M., Gao, H., Mondala, H., Bagnol, D., Unett, D., Liang, Y., Demarest, K., Semple, G., Behan, D. P., and Leonard, J. (2007) **A Role for β-Cell-Expressed GPR119 in Glycemic Control by Enhancing Glucose-Dependent Insulin Release.** *Endocrinology* **148 (6)**, 2601-2609.