Tango™ GPR119-*bla* U2OS DA cells

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Catalog Numbers – K1770 and K1777

Cell Line Descriptions

Tango™ GPR119-*bla* U2OS DA (Division Arrested) cells and Tango™ GPR119-*bla* U2OS cells contain the human G-protein Coupled Receptor 119 (GPR119) linked to a TEV protease site and a Gal4-VP16 transcription factor stably integrated into the Tango™ GPCR-*bla* U2OS parental cell line. This parental cell line stably expresses a beta-arrestin/TEV protease fusion protein and the beta-lactamase (*bla*) reporter gene under the control of a UAS 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 the Tango™ GPR119-*bla* U2OS cells and the Tango™ GPR119-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of AR231453 (1) (Figure 1). In addition, Tango™ GPR119-*bla* U2OS 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**

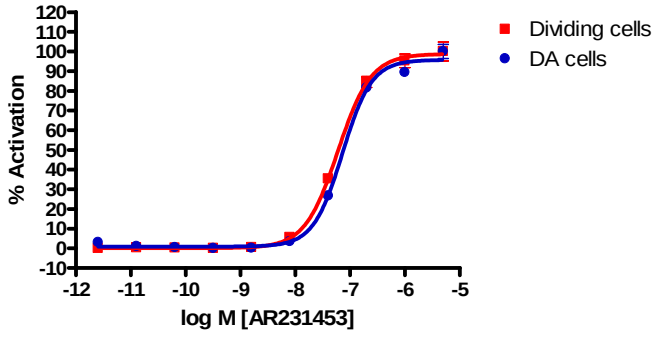
	DA cells	Dividing Cells
EC_{50}	72.4 nM	59.8 nM
Z'-factor	0.76	0.72
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 or 16 hrs
Max. [Stimulation]		= 1000 nM

Assay Testing Summary

2. **Assay performance with variable cell number.**
3. **Assay performance with variable stimulation time.**

Primary Agonist Dose Response

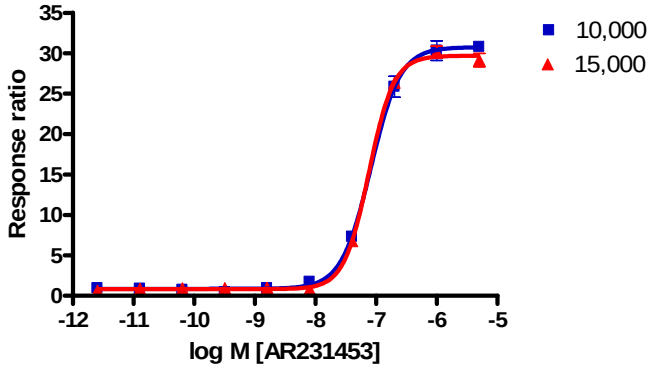
Figure 1 — Tango™ GPR119-*bla* U2OS cells and Tango™ GPR119-*bla* U2OS DA cells dose response to AR231453 under optimized conditions



Tango™ GPR119-*bla* U2OS cells and Tango™ GPR119-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format. Cells were stimulated with a dilution series of AR231453 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 AR231453.

Assay Performance with Variable Cell Number

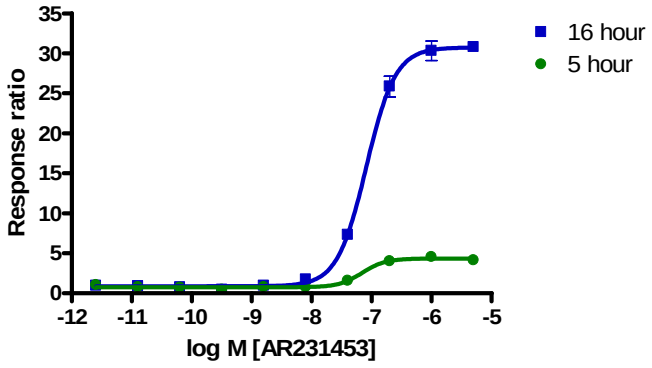
Figure 2 — Tango™ GPR119-*bla* U2OS cells dose response to AR231453 with 10K or 15K cells/ well



Tango™ GPR119-*bla* U2OS cells cells were plated in a 384-well format at 10,000 or 15,000 cells/well. Cells were stimulated with AR231453 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 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 3 – Tango™ GPR119-*bla* U2OS cells dose response to AR231453 with 5 or 16 hour stimulation times



GPR19-*bla* U2OS 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 5 or 16 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. Although the assay window is much larger at 16 hrs a suitable response is obtained at 5 hrs.

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.