Tango™ TBXA2R-*bla* U2OS DA Assay Kit

Tango™ TBXA2R-*bla* U2OS cells

Catalog Numbers – K1751 and K1549

Cell Line Descriptions

Tango™ TBXA2R-*bla* U2OS DA (Division Arrested) cells and Tango™ TBXA2R-*bla* U2OS cells contain the human Thromboxane A2 receptor (TBXA2R) 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. . Division Arrested (DA) cells are available as an Assay Kit, which includes cells and sufficient substrate to analyze 1 x 384-well plate.

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™ TBXA2R-*bla* U2OS cells and the Tango™ TBXA2R-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of U46619 (Figure 1). In addition, Tango™ TBXA2R-*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. U46619 dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	202 nM	191 nM
Z'-factor	0.78	0.51

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 or 16 hrs
Max. [Stimulation] = 1000 nM

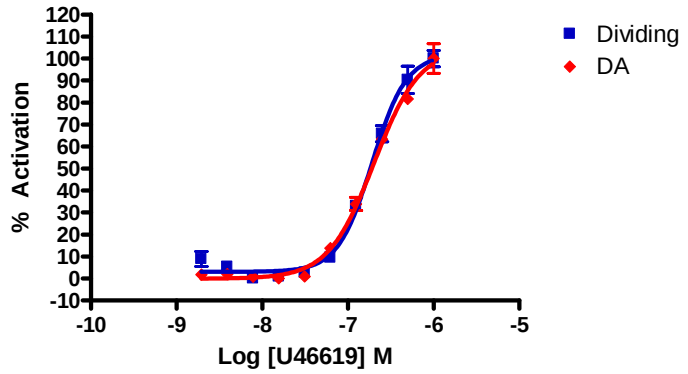
2. Antagonist dose response

L, 655,240 IC_{50} = 0.28 pM

3. Assay performance with variable stimulation time

Primary Agonist Dose Response

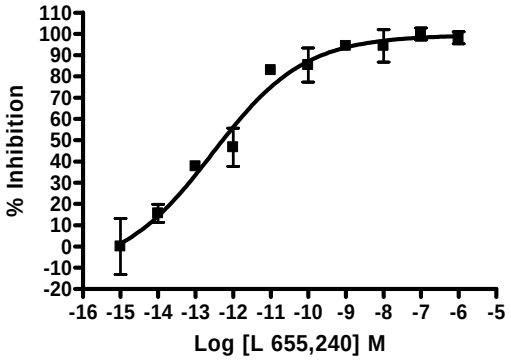
Figure 1 — Tango™ TBXA2R-*bla* U2OS cells and Tango™ TBXA2R-*bla* U2OS DA cells dose response to U46619 under optimized conditions



Tango™ TBXA2R-*bla* U2OS cells and Tango™ TBXA2R-*bla* U2OS DA 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 U46619 (Sigma D 8174) 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 U46619.

Antagonist Dose Response

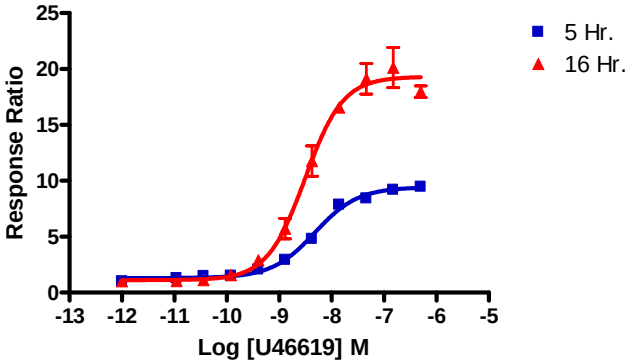
Figure 2 — Tango™ TBXA2R-*bla* U2OS cells dose response to L, 655,240



Tango™ TBXA2R-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to L, 655,240 (Sigma L 9539) for 30 min. and then stimulated with an EC80 concentration of U46619 (Sigma D 8174) 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 % Inhibition plotted against the indicated concentrations of L, 655,240.

Assay Performance with Variable Stimulation Time

Figure 6 – Tango™ TBXA2R-*bla* U2OS cells dose response to U46619 with 5 or 16 hour stimulation times



Tango™ TBXA2R-*bla* U2OS cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. U46619 (Sigma D 8174) 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 U46619.