Tango™ AGTRL1-*bla* U2OS DA Assay Kit

Tango™ AGTRL1-*bla* U2OS cells

Catalog Numbers – K1762 and K1754

Cell Line Descriptions

Tango™ AGTRL1-*bla* U2OS DA (Division Arrested) cells and Tango™ AGTRL1-*bla* U2OS cells contain the human angiotensin II receptor-like 1 (AGTRL1) 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 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™ AGTRL1-*bla* U2OS cells and the Tango™ AGTRL1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of Apellin-13 (Figure 1).

Validation Summary

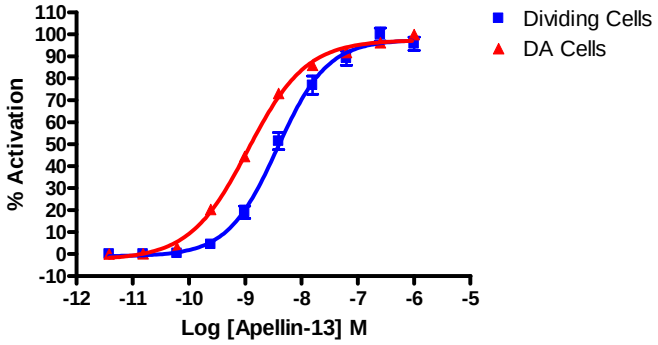
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. Apellin-13 dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1.1 nM	3.7 nM
Z'-factor	0.80	0.73
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 1000 nM

Primary Agonist Dose Response

Figure 1 — Tango™ AGTRL1-*bla* U2OS cells and Tango™ AGTRL1-*bla* U2OS DA cells dose response to Apellin-13 under optimized conditions



Tango™ AGTRL1-*bla* U2OS cells and Tango™ AGTRL1-*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 Apellin-13 (Sigma A6469) 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 Apellin-13.