Tango™ NPY5R-*bla* U2OS DA Assay Kit

Tango™ NPY5R-*bla* U2OS cells

Catalog Numbers – K1774 and K1782

Cell Line Descriptions

Tango™ NPY5R-*bla* U2OS DA (Division Arrested) cells and Tango™ NPY5R-*bla* U2OS cells contain the human Neuropeptide Y Receptor Y5 (NPY5R) 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 in 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™ NPY5R-*bla* U2OS cells and the Tango™ NPY5R-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of a Neuropeptide-Y (Figure 1). In addition, Tango™ NPY5R-*bla* U2OS cells have been tested for assay performance under variable conditions.

Validation Summary

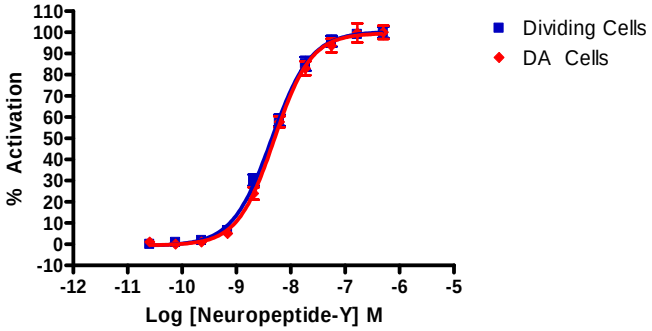
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. Neuropeptide-Y dose response under optimized conditions

	DA Cells	Dividing Cells
EC_{50}	4.96 nM	4.45 nM
Z'-factor	0.74	0.78
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 500 nM

Primary Agonist Dose Response

Figure 1 — Tango™ NPY5R-*bla* U2OS cells and Tango™ NPY5R-*bla* U2OS DA cells dose response to Neuropeptide-Y under optimized conditions



Tango™ NPY5R-*bla* U2OS cells and Tango™ NPY5R-*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 Neuropeptide-Y (Tocris 1153) 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 Neuropeptide-Y.