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**Optimization of the Tango™ NPY1R-*bla* U2OS Cell Line**

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**Tango™ NPY1R-*bla* U2OS cells**

Catalog Numbers – K1803

**Cell Line Descriptions**

NPY1R-*bla* U2OS cells contain the human Neuropeptide Y Receptor Y1 (NPY1R) 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.

The Tango™ NPY1R-*bla* U2OS cells have been functionally validated for Z' factor and EC<sub>50</sub> concentrations of Neuropeptide-Y (Figure 1). In addition, Tango™ NPY1R-*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

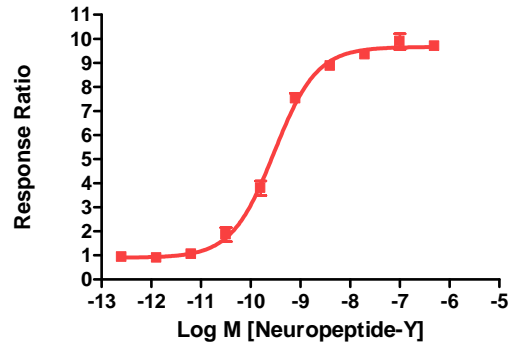
	<u>Dividing Cells</u>
EC <sub>50</sub>	0.3 nM
Z'-factor	0.82
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 500 nM

### 2. Antagonist dose response

<b>GR231118</b>	
Dividing IC <sub>50</sub>	= 31 nM
Cryopreserved IC <sub>50</sub>	= 16 nM
Division Arrested IC <sub>50</sub>	= 8.8 nM

## Primary Agonist Dose Response

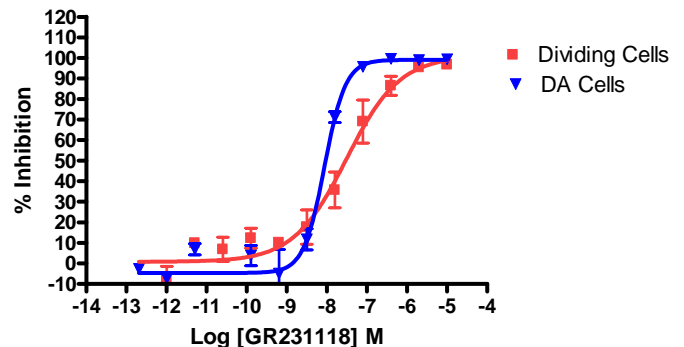
Figure 1 — Tango™ NPY1R-*bla* U2OS cells dose response to Neuropeptide-Y under optimized conditions



Tango™ NPY1R-*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.

## Antagonist Dose Response

Figure 3 — Tango™ NPY1R-*bla* U2OS cells dose response to GR231118



Tango™ NPY1R-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to GR231118 (Sigma N8648) for 30 min. and then stimulated with an EC<sub>80</sub> concentration 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of GR231118.