**Tango™ NPY2R-*bla* U2OS DA cells**

**Tango™ NPY2R-*bla* U2OS cells**

Catalog Numbers – K1501 and K1479

## Cell Line Descriptions

Tango™ NPY2R-*bla* U2OS DA (Division Arrested) cells and Tango™ NPY2R-*bla* U2OS cells contain the human Neuropeptide Y Receptor Y2 (NPY2R) 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 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™ NPY2R-*bla* U2OS cells and the Tango™ NPY2R-*bla* U2OS DA cells have been functionally validated for Z' factor and $EC_{50}$ concentrations of Neuropeptide Y (Figure 1). In addition, Tango™ NPY2R-*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 |
|---|---|---|
| $EC_{50}$ | 6.5 nM | 10.6 nM |
| Z'-factor | 0.77 | 0.78 |
| Recommended cell no. /well | | = 10,000 |
| Recommended Stim. Time | | = 5 hrs |
| Max. [Stimulation] | | = 1000 nM |

2. **Assay performance with variable assay media.**

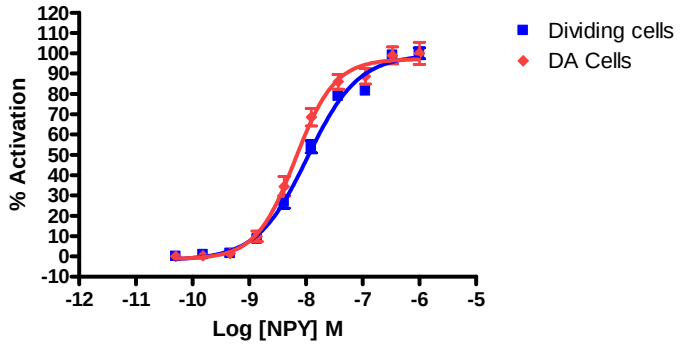
   See graph below

3. **Antagonist dose response**

   BIIE 0246 $IC_{50}$ = 2.0 nM

## Primary Agonist Dose Response

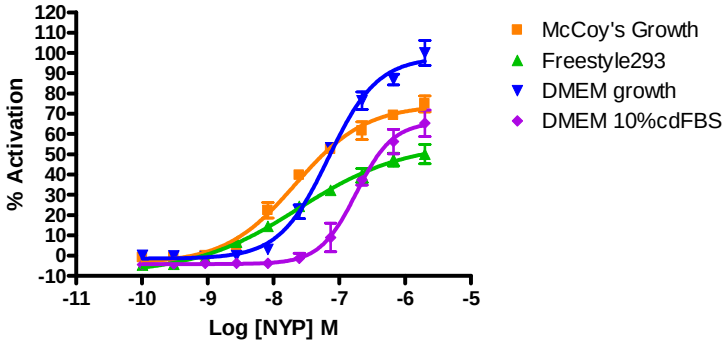
**Figure 1 — Tango™ NPY2R-*bla* U2OS cells and Tango™ NPY2R-*bla* U2OS DA cells dose response to Neuropeptide Y under optimized conditions**




Tango™ NPY2R-*bla* U2OS cells and Tango™ NPY2R-*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 (Sigma N-5017) 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.

## Assay Performance with Variable Assay Media.

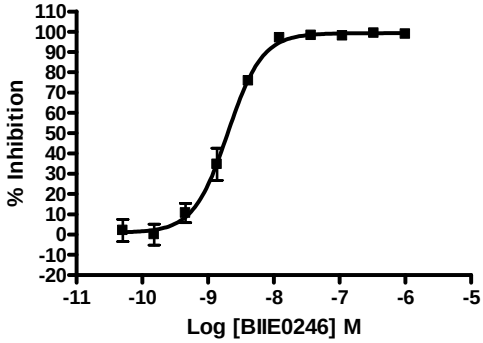
**Figure 2 – Tango™ NPY2R-*bla* U2OS cells dose response to Neuropeptide Y with McCoy's Growth Media, Freestyle 293 Media, DMEM Growth Media or DMEM + 10% cdFBS.**




Tango™ NPY2R-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 16-24 hours in varying assay media; McCoy's Growth Media, Freestyle 293 Media, DMEM Growth Media or DMEM with 10%cdFBS. Cells were stimulated with a dilution series of Neuropeptide Y (Sigma N-5017) 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™ NPY2R-*bla* U2OS cells dose response to BIIE 0246**




Tango™ NPY2R-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to BIIE 0246 (Tocris 1700) for 30 min. and then stimulated with an $EC_{80}$ concentration of Neuropeptide Y (Sigma N-5017) 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 BIIE 0246.