Tango™ GPR8-*bla* U2OS DA cells

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Catalog Numbers – K1497and K1478

Cell Line Descriptions

Tango™ GPR8-*bla* U2OS DA (Division Arrested) cells and Tango™ GPR8-*bla* U2OS cells contain the human Neuropeptides B/W receptor 2 (GPR8) 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.

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™ GPR8-*bla* U2OS cells and the Tango™ GPR8-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of Neuropeptide B-29 (NPB29) (Figure 1). In addition, Tango™ GPR8-*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.

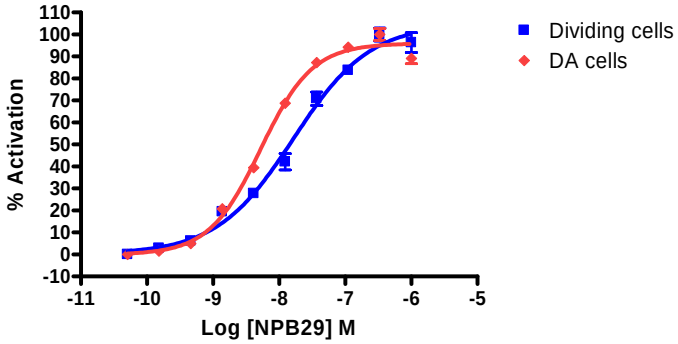
1. **Neuropeptide B-29 dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	5.3 nM	16.3 nM
Z'-factor	0.75	0.60
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 1000 nM

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

Primary Agonist Dose Response

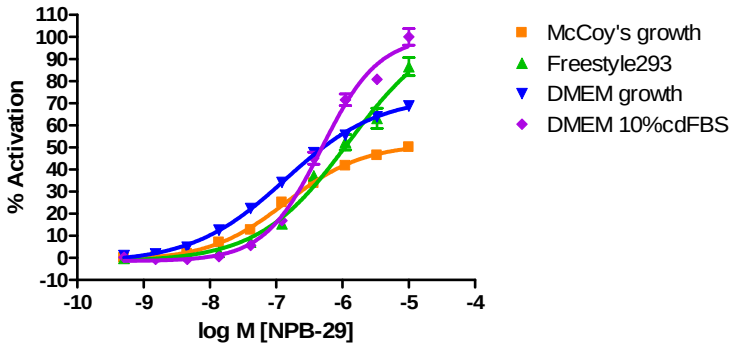
Figure 1 — Tango™ GPR8-*bla* U2OS cells and Tango™ GPR8-*bla* U2OS DA cells dose response to Neuropeptide B-29 under optimized conditions



Tango™ GPR8-*bla* U2OS cells and Tango™ GPR8-*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 B-29 (Phoenix Pharm. 005-51) 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 B-29.

Assay Performance with Variable Assay Media.

Figure 2 — Tango™ GPR8-*bla* U2OS cells dose response to Neuropeptide B-29with McCoy's Growth Media, Freestyle 293 Media, DMEM Growth Media and DMEM + 10% cd FBS



Tango™ GPR8-*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. On the day of the assay, cells were stimulated with Neuropeptide B-29 (Phoenix Pharm. 005-51) in the presence of 0.1% DMSO for 5hours. 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 B-29.