Tango™ MTNR1B-*bla* U2OS DA cells

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Catalog Numbers – K1755 and K1753

Cell Line Descriptions

Tango™ MTNR1B-*bla* U2OS DA (Division Arrested) cells and Tango™ MTNR1B-*bla* U2OS cells contain the human Melatonin Receptor 1B (MTNR1B) 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.

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™ MTNR1B-*bla* U2OS cells and the Tango™ MTNR1B-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of Melatonin (Figure 1). In addition, Tango™ MTNR1B-*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. Melatonin dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	21.81 nM	27.85 nM
Z'-factor	0.84	0.90
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 or 16 hrs
Max. [Stimulation]		= 400000 nM

2. Alternate agonist dose response

IIK7 EC_{50} = 132.5 nM

3. Antagonist dose response

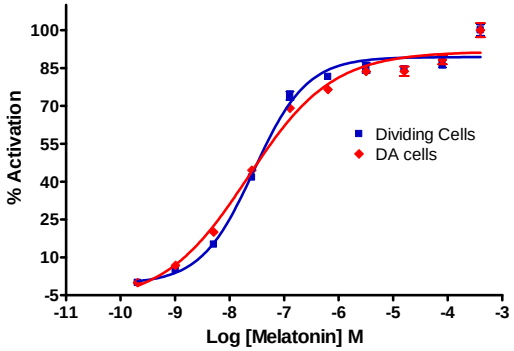
K185 (Dividing) IC_{50} = 47.79 µM
K185 (DA) IC_{50} = 52.26 µM

4. Assay performance with variable stimulation time.

5 Hr. EC_{50} = 19.1 nM
16 Hr. EC_{50} = 20.7 nM

Primary Agonist Dose Response

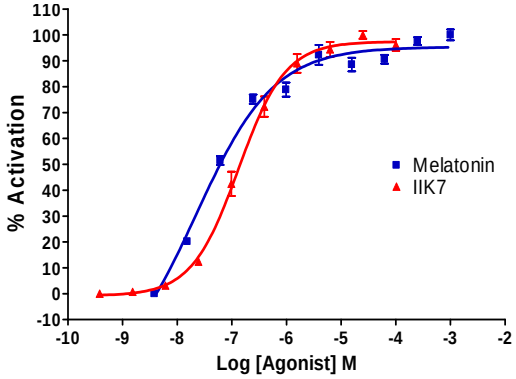
Figure 1 — Tango™ MTNR1B-*bla* U2OS cells and Tango™ MTNR1B-*bla* U2OS DA cells dose response to Melatonin under optimized conditions



Tango™ MTNR1B-*bla* U2OS cells Tango™ MTNR1B-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of Melatonin (Sigma M5250) in the presence of 0.1% DMSO for 16 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 Melatonin.

Alternate Agonist Dose Response and Selectivity

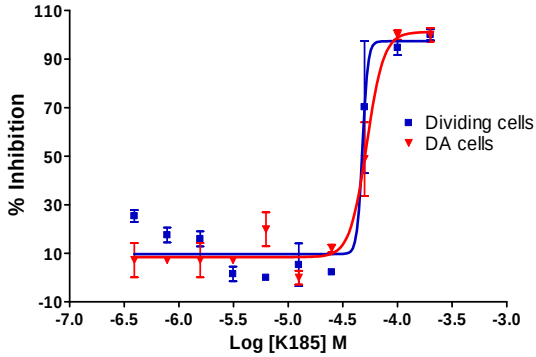
Figure 2 — Tango™ MTNR1B-*bla* U2OS cells dose response to Melatonin and IIK7.



Tango™ MTNR1B-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and stimulated with Melatonin (Sigma M5250) or IIK7 (Sigma, I5531) over the indicated concentration range in the presence of 0.1% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

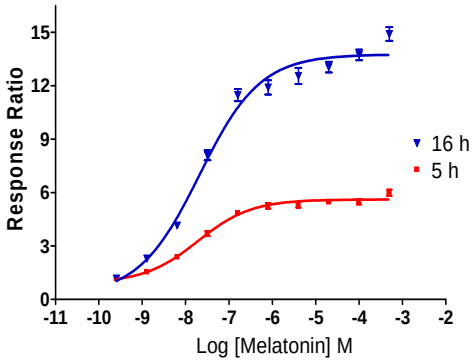
Figure 3 — Tango™ MTNR1B-*bla* U2OS cells dose response to K185



Tango™ MTNR1B-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to K185 (Sigma K1888) for 30 min. and then stimulated with an EC80 concentration of Melatonin (Sigma M5250) 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 K185.

Assay Performance with Variable Stimulation Time

Figure 4 – Tango™ MTNR1B-*bla* U2OS cells dose response to Melatonin with 5 or 16 hour stimulation times



MTNR1B-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well plate and incubated for 16-24 hours. Melatonin (Sigma M5250) in 0.1% DMSO was either added at the time of plating (for the 16 hour assay) or was added for 5 hours after the overnight incubation (for the 5 hour assay). The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 ratio plotted against the indicated concentrations of Melatonin.