Tango™ OPRM1-*bla* U2OS DA Assay Kit

Tango™ OPRM1-*bla* U2OS cells

Catalog Numbers – K1599 and K1523

Cell Line Descriptions

Tango™ OPRM1-*bla* U2OS DA (Division Arrested) cells and Tango™ OPRM1-*bla* U2OS cells contain the human Opioid Receptor Mu 1 (OPRM1) 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™ OPRM1-*bla* U2OS cells and the Tango™ OPRM1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of DAMGO (Figure 1). In addition, Tango™ OPRM1-*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. DAMGO dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	4.6 nM	5.22 nM
Z'-factor	0.76	0.74
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 16-24 hrs
Max. [Stimulation]		= 3000 nM

2. Antagonist dose response

β-FNA IC_{50}= 2.79 nM

Assay Testing Summary

3. Assay performance with variable assay media.

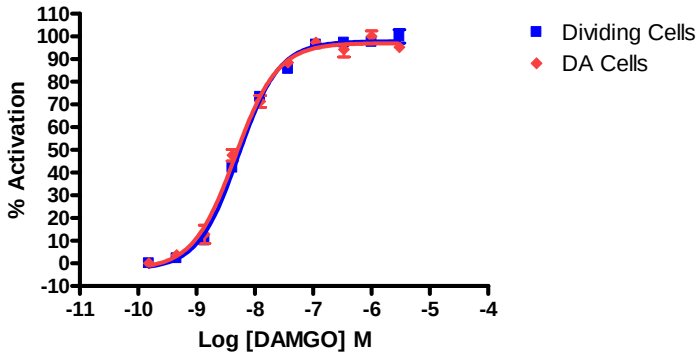
See graph below

4. Assay performance with variable stimulation time.

See graph below

Primary Agonist Dose Response

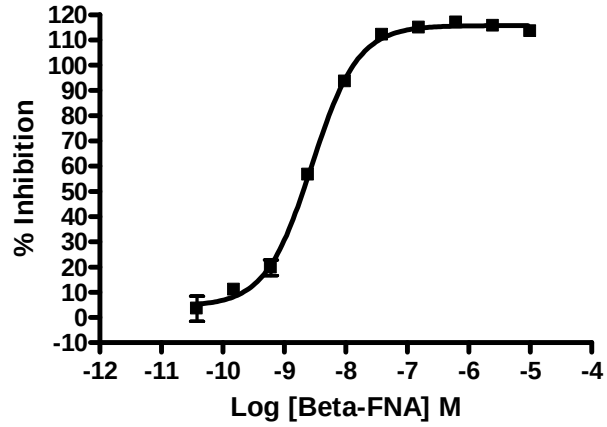
Figure 1 — Tango™ OPRM1-*bla* U2OS cells and Tango™ OPRM1-*bla* U2OS DA cells dose response to DAMGO under optimized conditions



Tango™ OPRM1-*bla* U2OS cells and Tango™ OPRM1-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format. Cells were stimulated with a dilution series of DAMGO (Sigma E7384) in the presence of 0.1% DMSO for 16-24 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 DAMGO.

Antagonist Dose Response

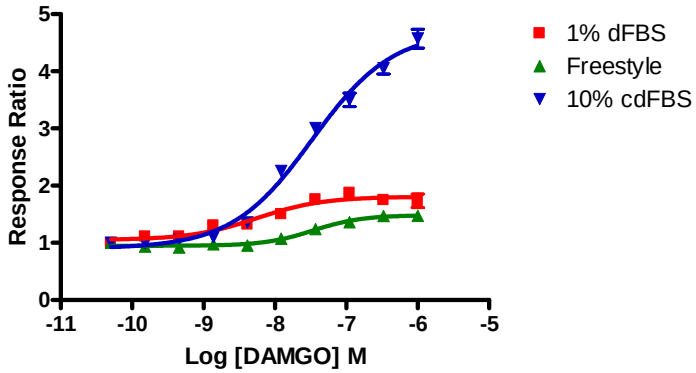
Figure 2 — Tango™ OPRM1-*bla* U2OS cells dose response to β-FNA



Tango™ OPRM1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format. Cells were exposed to β-FNA (Sigma O003) for 30 min. and then stimulated with an EC_{80} concentration of DAMGO (Sigma E7384) in the presence of 0.1% DMSO for 16-24 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 β-FNA.

Assay Performance with Variable Assay Media

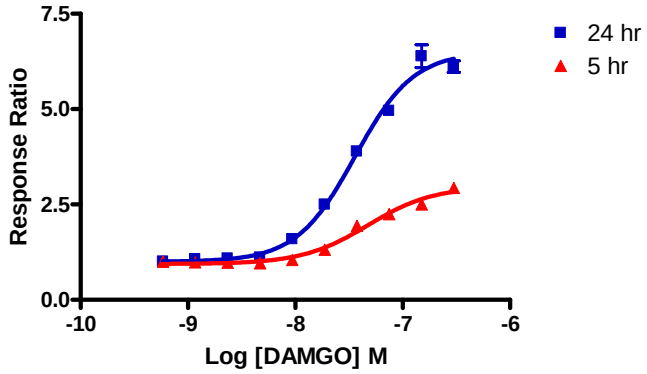
Figure 3 — Tango™ OPRM1-*bla* U2OS cells dose response to DAMGOwith with McCoy's Growth Media, Freestyle 293 Media, or DMEM + 10% cdFBS.



Tango™ OPRM1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate in varying assay media; McCoy's Growth Media, Freestyle 293 Media, or DMEM with 10%cdFBS. Cells were stimulated with DAMGO (Sigma E7384) in the presence of 0.1% DMSO for 16-24 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 Response Ratio plotted for each replicate against the concentrations of DAMGO.

Assay Performance with Variable Stimulation Time

Figure 4 – Tango™ OPRM1-*bla* U2OS cells dose response to DAMGO with 5 or 16 hour stimulation times



Tango™ OPRM1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate. DAMGO (Sigma E7384) was either added at the time of plating (for the 16 hour assay) or was added to 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 Response Ratio plotted for each replicate against the concentrations of DAMGO.