Tango™ OPRD1-*bla* U2OS DA Assay Kit

Tango™ OPRD1-*bla* U2OS cells

Catalog Numbers – K1771 and K1778

Cell Line Descriptions

Tango™ OPRD1-*bla* U2OS DA (Division Arrested) cells and Tango™ OPRD1-*bla* U2OS cells contain the human Opioid receptor, delta 1 (OPRD1) 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. Division Arrested (DA) cells are available as 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™ OPRD1-*bla* U2OS cells and the Tango™ OPRD1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of SNC80 (Figure 1). In addition, Tango™ OPRD1-*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. SNC80 dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	21.1 nM	18.64 nM
Z'-factor	0.70	0.82
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 16 hrs
Max. [Stimulation]		= 10000 nM

2. Alternate agonist dose response

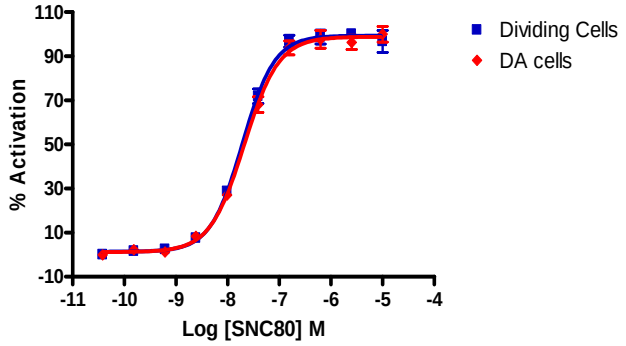
DPDPE EC_{50} = 86.1 nM

3. Antagonist dose response

SDM25N IC_{50}= 1.31 nM

Primary Agonist Dose Response

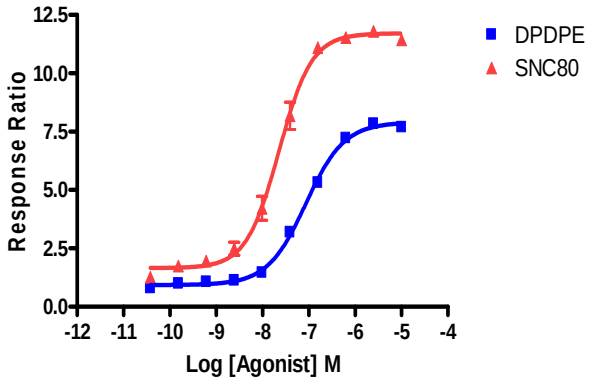
Figure 1 — Tango™ OPRD1-*bla* U2OS cells and Tango™ OPRD1-*bla* U2OS DA cells dose response to SNC80 under optimized conditions



Tango™ OPRD1-*bla* U2OS cells and Tango™ OPRD1-*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 SNC80 (Sigma S2812) 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 SNC80.

Alternate Agonist Dose Response and Selectivity

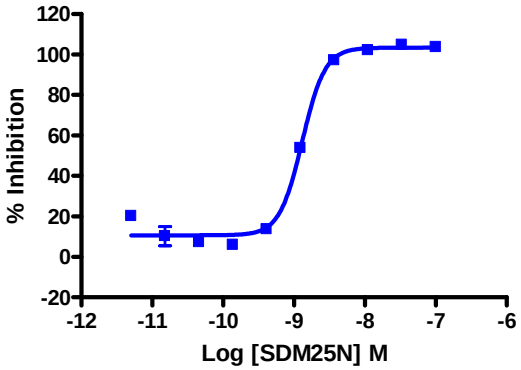
Figure 2 — Tango™ OPRD1-*bla* U2OS cells dose response to SNC80 and DPDPE



Tango™ OPRD1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with SNC80 (Sigma S2812) and DPDPDE (Sigma, E3888) 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 Response ratio plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

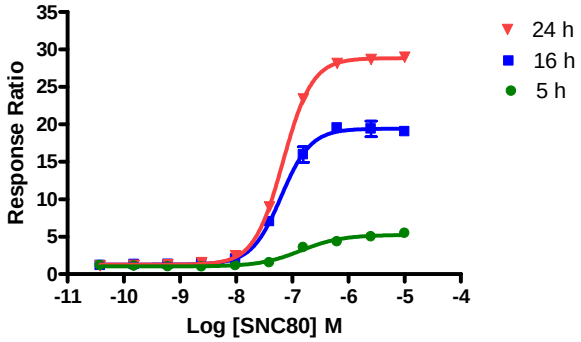
Figure 3 — Tango™ OPRD1-*bla* U2OS cells dose response to SDM25N



Tango™ OPRD1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to SDM25N (Tocris 1410) for 30 min. and then stimulated with an EC80 concentration of SNC80 (Sigma S2812) 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of SDM25N.

Assay Performance with Variable Stimulation Time

Figure 4 – Tango™ OPRD1-*bla* U2OS cells dose response to SNC80 with 5, 16 or 24 hour stimulation times



Tango™ OPRD1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 16-24 hours. SNC80 (Sigma S2812) 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 the Response Ratios plotted against the indicated concentrations of SNC80.