
Optimization of the Tango™ OPRL1-*bla* U2OS Cell Line

Tango™ OPRL1-*bla* U2OS DA cells**Tango™ OPRL1-*bla* U2OS cells**

Catalog Numbers – K1792 and K1786

Cell Line Descriptions

Tango™ OPRL1-*bla* U2OS cells and Tango™ OPRL1-*bla* U2OS DA cells contain the human Opioid Receptor-Like 1 (OPRL1) 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™ OPRL1-*bla* U2OS cells and the Tango™ OPRL1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC₅₀ concentrations of U-50,488 (Figure 1)

Validation Summary

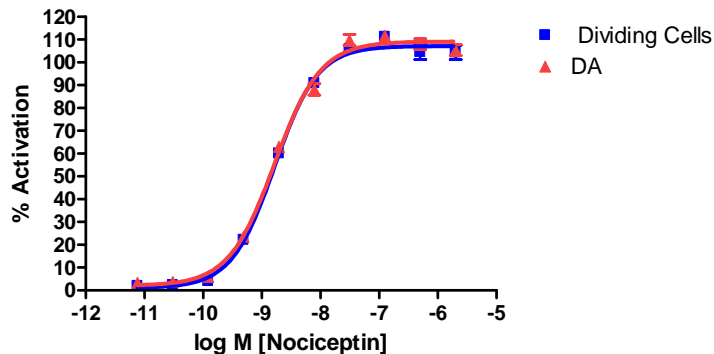
Testing and validation of this assay was evaluated in a 384-well format using LiveBLazer™-FRET B/G Substrate.

1. Orphanin FQ dose response under optimized conditions

| | |
|----------------------------|-----------------------|
| | <u>Dividing Cells</u> |
| EC ₅₀ | 1.64 nM |
| Z'-factor | 0.76 |
| Recommended cell no. /well | = 10,000 |
| Recommended Stim. Time | = 5 hrs |
| Max. [Stimulation] | = 2000 nM |

Primary Agonist Dose Response

Figure 1 — Tango™ OPRL1-bla U2OS and Tango™ OPRL1-bla U2OS DA cell dose response to Orphanin FQ under optimized conditions



Tango™ OPRL1-bla U2OS cells and Tango™ OPRL1-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 Orphanin FQ (Tocris 910) 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 Orphanin FQ.