Tango™ HTR1D-*bla* U2OS DA cells

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Catalog Numbers –K1611 and K1524

Cell Line Descriptions

Tango™ HTR1D-*bla* U2OS DA (Division Arrested) cells and Tango™ HTR1D-*bla* U2OS cells contain the human 5-hydroxytryptamine (serotonin) receptor 1D (HTR1D) 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™ HTR1D-*bla* U2OS cells and the Tango™ HTR1D-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of 5-Hydroxytryptamine (Figure 1). In addition, Tango™ HTR1D-*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. 5-Hydroxytryptamine dose response under optimized conditions

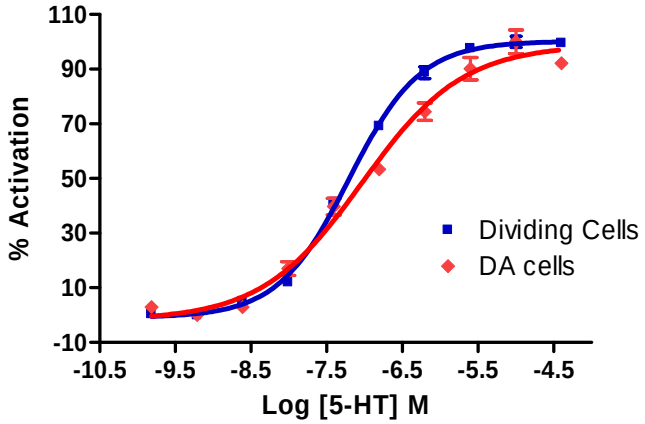
	DA cells	Dividing Cells
EC_{50}	93 nM	62 nM
Z'-factor	0.77	0.92
Recommended cell no. /well	= 10,000	
Recommended Stim. Time	= 16 hrs	
Max. [Stimulation]	= 40,000 nM	

2. Antagonist dose response

BRL 15572 (Dividing) IC_{50} = 260.3 µM
BRL 15572 (DA) IC_{50} = 479.8 µM

Primary Agonist Dose Response

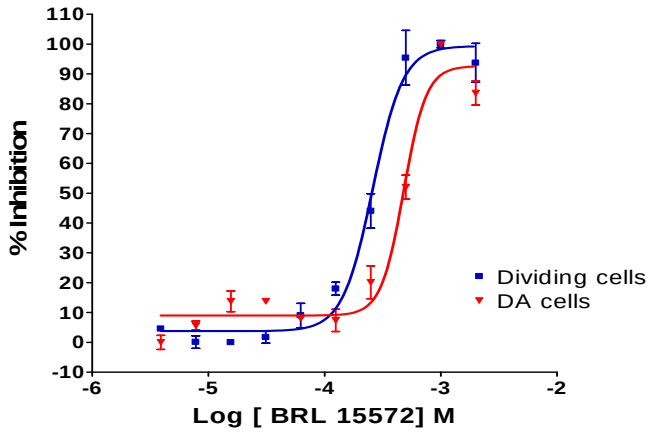
Figure 1 — Tango™ HTR1D-*bla* U2OS cells and Tango™ HTR1D-*bla* U2OS DA cells dose response to 5-Hydroxytryptamine under optimized conditions



Tango™ HTR1D-*bla* U2OS cells and Tango™ HTR1D-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of 5-Hydroxytryptamine (Sigma H9523) 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 5-Hydroxytryptamine.

Antagonist Dose Response

Figure 2 — Tango™ HTR1D-*bla* U2OS cells dose response to BRL 15572



Tango™ HTR1D-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to BRL 15572 (Sigma B9929) for 30 min. and then stimulated with an EC80 concentration of 5-Hydroxytryptamine (Sigma H9523) 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 % Inhibition plotted against the indicated concentrations of BRL 15572.