Tango™ CXCR2-*bla* U2OS DA Assay Kit

Tango™ CXCR2-*bla* U2OS cells

Catalog Numbers – K1769 and K1521

Cell Line Descriptions

Tango™ CXCR2-*bla* U2OS DA (Division Arrested) cells and Tango™ CXCR2-*bla* U2OS cells contain the human Chemokine (C-X-C motif) receptor 2 (CXCR2) 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™ CXCR2-*bla* U2OS cells and the Tango™ CXCR2-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of IL-8 (Figure 1). In addition, Tango™ CXCR2-*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. IL-8 dose response under optimized conditions

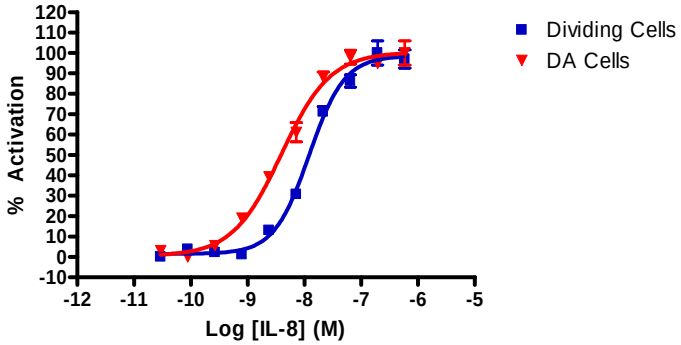
	DA cells	Dividing Cells
EC_{50}	3.9 nM	12.1 nM
Z'-factor	0.74	0.67
Recommended cell no. /well	= 10,000	
Recommended Stim. Time	= 5 hrs	
Max. [Stimulation]	= 290 nM	

2. Antagonist dose response

not currently available

Primary Agonist Dose Response

Figure 1 — Tango™ CXCR2-*bla* U2OS cells and Tango™ CXCR2-*bla* U2OS DA cells dose response to IL-8 under optimized conditions



Tango™ CXCR2-*bla* U2OS cells and Tango™ CXCR2-*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 IL-8 (Biosource (IVGN) PHC0885) 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 IL-8.