Tango™ CCR1-*bla* U2OS DA Assay Kit

Tango™ CCR1-*bla* U2OS cells

Catalog Numbers – K1793 and K1787

Cell Line Descriptions

Tango™ CCR1-*bla* U2OS DA (Division Arrested) cells and Tango™ CCR1-*bla* U2OS cells contain the human Chemokine (C-C Motif) Receptor 1 (CCR1) 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™ CCR1-*bla* U2OS cells and the Tango™ CCR1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of MIP-1a (Figure 1). In addition, Tango™ CCR1-*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. MIP-1a dose response under optimized conditions

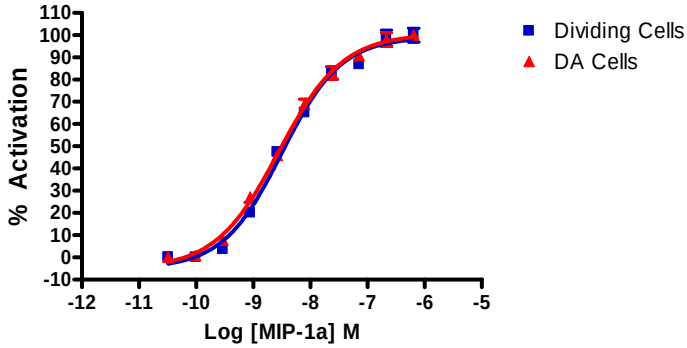
	DA cells	Dividing Cells
EC_{50}	3.2 nM	2.8 nM
Z'-factor	0.83	0.72
Recommended cell no. /well	= 10,000	
Recommended Stim. Time	= 5 hrs	
Max. [Stimulation]	= 641 nM	

2. Antagonist dose response

vMIP II IC_{50}	= 78 nM

Primary Agonist Dose Response

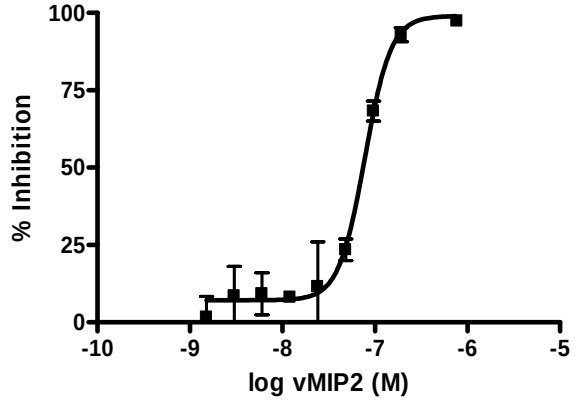
Figure 1 — Tango™ CCR1-*bla* U2OS cells and Tango™ CCR1-*bla* U2OS DA cells dose response to MIP-1a under optimized conditions



Tango™ CCR1-*bla* U2OS cells and Tango™ CCR1-*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 MIP-1a (Biosource (IVGN) PHC1105) 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 MIP-1a.

Antagonist Dose Response

Figure 3 — Tango™ CCR1-*bla* U2OS cells dose response to vMIP II



Tango™ CCR1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to vMIP II (R&D systems 601-VB) for 30 min. and then stimulated with an EC80 concentration of MIP-1a (Biosource (IVGN) PHC1105) 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of vMIP II.