Tango™ CCR5-*bla* U2OS cells

Catalog Numbers – K1788

Cell Line Descriptions

The Tango™ CCR5-*bla* U2OS cells contain the human Chemokine (C-C Motif) Receptor 5 (CCR5) 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.

The Tango™ CCR5-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of MIP-1a (Figure 1). In addition, Tango™ CCR5-*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**

	Dividing Cells
EC_{50}	18.5 nM
Z'-factor	0.89
Recommended cell no. /well	=10,000
Recommended Stim. Time	=16 hrs
Max. [Stimulation]	=641 nM

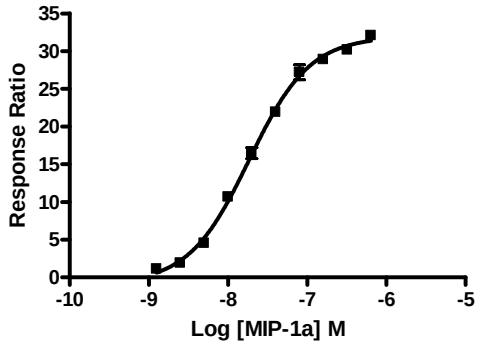
2. **Assay performance with variable cell number.**

3. **Antagonist dose response**

IC_{50} = 324 nM

Primary Agonist Dose Response

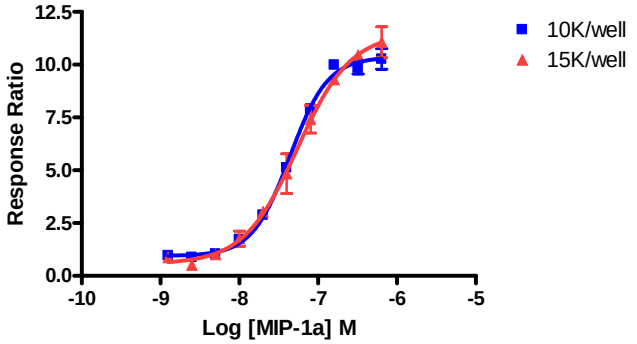
Figure 1 — Tango™ CCR5-*bla* U2OS cells dose response to MIP-1a under optimized conditions



Tango™ CCR5-*bla* U2OS 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 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 Response Ratios plotted for each replicate against the concentrations of MIP-1a.

Assay Performance with Variable Cell Number

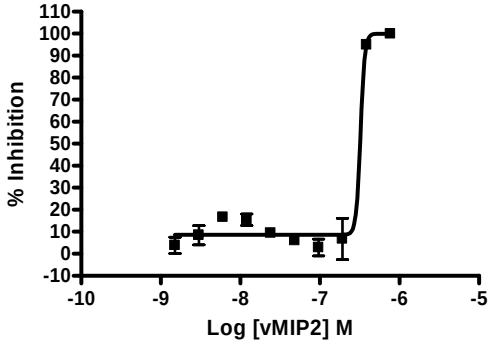
Figure 2 — Tango™ CCR5-*bla* U2OS cells dose response to MIP-1a with 10K or 15K cells/well



Tango™ CCR5-*bla* U2OS cells cells were plated in a 384-well format at 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with MIP-1a (Biosource (IVGN) PHC1105) in the presence of 0.1% DMSO for 16hours. 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 Response Ratios plotted for each replicate against the concentrations of MIP-1a.

Antagonist Dose Response

Figure 3 — Tango™ CCR5-*bla* U2OS cells dose response to vMIP2



Tango™ CCR5-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to vMIP2 for 30 min. and then stimulated with an EC80 concentration of MIP-1a (Biosource (IVGN) PHC1105) 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 not currently available.