Tango™ CCR2-*bla* U2OS cells

Catalog Numbers – K1809

Cell Line Descriptions

Tango™ CCR2-*bla* U2OS cells contain the human Chemokine (C-C Motif) Receptor 2 (CCR2) 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™ CCR2-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of MCP-1 (Figure 1). In addition, Tango™ CCR2-*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. **MCP-1 dose response under optimized conditions**

	Dividing Cells
EC_{50}	6.7 nM
Z'-factor	0.75
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 625 nM

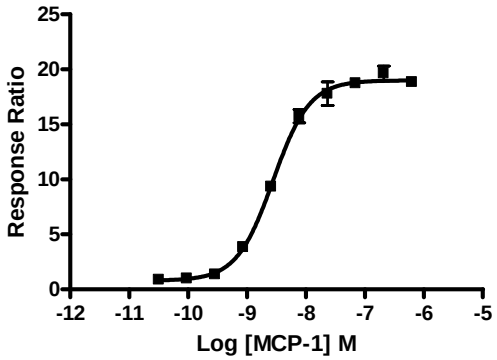
2. **Antagonist dose response**

vMIP II IC_{50}= 145 nM

3. **Assay performance with variable stimulation time.**

Primary Agonist Dose Response

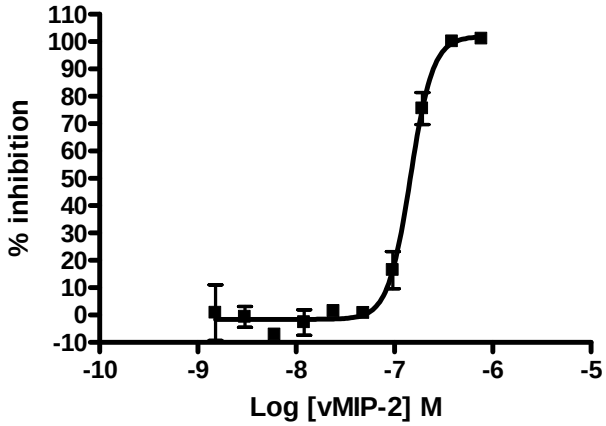
Figure 1 — Tango™ CCR2-*bla* U2OS cells and Tango™ CCR2-*bla* U2OS DA cells dose response to MCP-1 under optimized conditions



Tango™ CCR2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of MCP-1 (Biosource (IVGN) PHC1011) 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 MCP-1.

Antagonist Dose Response

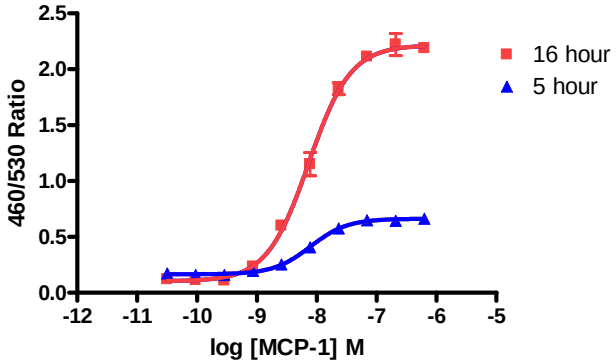
Figure 2 — Tango™ CCR2-*bla* U2OS cells dose response to vMIP II



Tango™ CCR2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format, exposed to vMIP II (R&D systems 601-VB) for 30 minutes, and then stimulated with an EC80 concentration of MCP-1 (Biosource (IVGN) PHC1011) 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 vMIP II.

Assay Performance with Variable Stimulation Time

Figure 3 – Tango™ CCR2-*bla* U2OS cells dose response to MCP-1 with 5 or 16 hour stimulation times



Tango™ CCR2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 16-24 hours. MCP-1 (Biosource (IVGN) PHC1011) was either added at the time of plating (for the 16 hour assay) or was added to for 5 hours after the overnight incubation (for the 5 hour assay). The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the blue/green ratios plotted against the indicated concentrations of MCP-1.