Tango™ CXCR3-*bla* U2OS cells

Catalog Numbers – K1829

Cell Line Descriptions

Tango™ CXCR3-*bla* U2OS cells contain the human chemokine (C-X-C motif) receptor 3 (CXCR3) 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™ CXCR3-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of I-TAC (CXCL11) (Figure 1). In addition, Tango™ CXCR3-*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. I-TAC (CXCL11) dose response under optimized conditions

	Dividing Cells
EC_{50}	4.29 nM
Z'-factor	0.90
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 500 nM

2. Alternate agonist dose response

CXCL10 EC_{50} = 227 nM

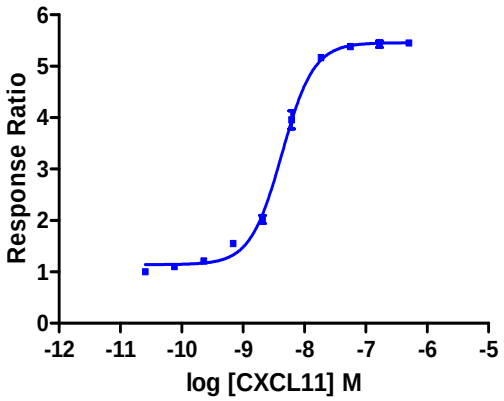
3. Antagonist dose response

vMIP II IC_{50}= 519.5 nM

4. Assay Performance with Variable Stimulation Time

Primary Agonist Dose Response

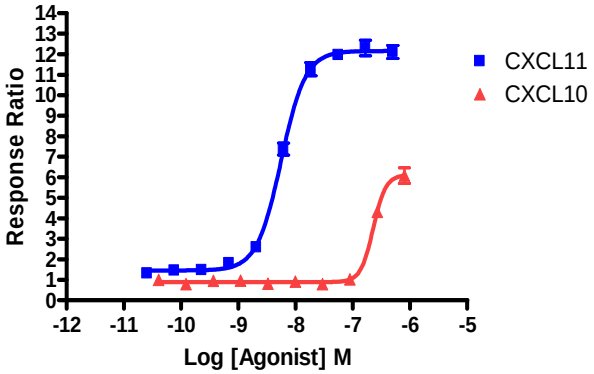
Figure 1 — Tango™ CXCR3-*bla* U2OS cells dose response to I-TAC (CXCL11) under optimized conditions



Tango™ CXCR3-*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 I-TAC (CXCL11) (Biosource (IVGN) PHC1691) 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 I-TAC (CXCL11).

Alternate Agonist Dose Response

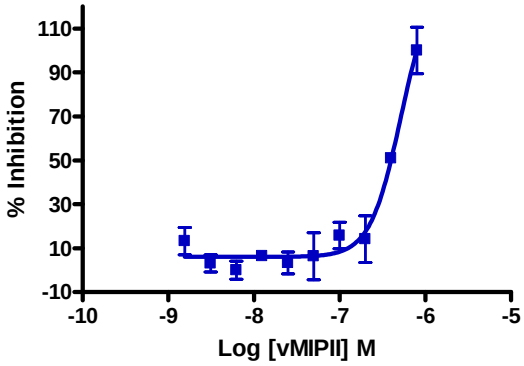
Figure 2 — Tango™ CXCR3-*bla* U2OS cells dose response to CXCL11 and CXCL10.



Tango™ CXCR3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and stimulated with CXCL11 (IVGN (Biosource) PHC 1691) or CXCL10 (IVGN(Biosource) PHC 1085) over the indicated concentration range in the presence of 0.1% DMSO for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the response ratio plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

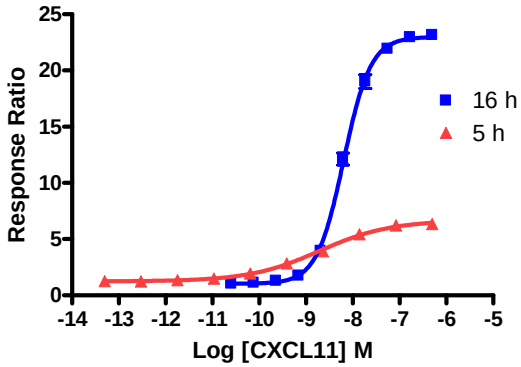
Figure 3 — Tango™ CXCR3-*bla* U2OS cells dose response to vMIP II



Tango™ CXCR3-*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 EC_{80} concentration of I-TAC (CXCL11) (Biosource (IVGN) PHC1691) 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 4 – Tango™ CXCR3-*bla* U2OS cells dose response to I-TAC (CXCL11) with 5 or 16 hour stimulation times



Tango™ CXCR3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 16-24 hours. I-TAC (CXCL11) (Biosource (IVGN) PHC1691) 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 Response Ratios plotted against the indicated concentrations of I-TAC (CXCL11).