
Optimization of the Tango™ CXCR7-*bla* U2OS Cell Line

Tango™ CXCR7-*bla* U2OS cells

Catalog Numbers – K1832

Cell Line Descriptions

The Tango™ CXCR7-*bla* U2OS cells contain the human Chemokine (C-X-C motif) receptor 7 (CXCR7) 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™ CXCR7-*bla* U2OS cells have been functionally validated for Z' factor and EC₅₀ concentrations of SDF1a (Figure 1). In addition, Tango™ CXCR7-*bla* U2OS cells have been tested for assay performance under variable conditions.

Validation Summary

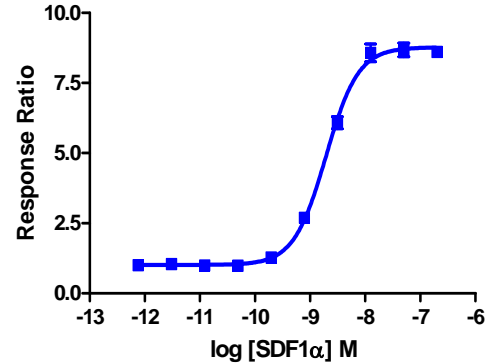
Testing and validation of this assay was evaluated in a 384-well format using LiveBLazer™-FRET B/G Substrate.

1. SDF1a dose response under optimized conditions

	<u>Dividing Cells</u>
EC ₅₀	1.94 nM
Z'-factor	0.80
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 200 nM

Primary Agonist Dose Response

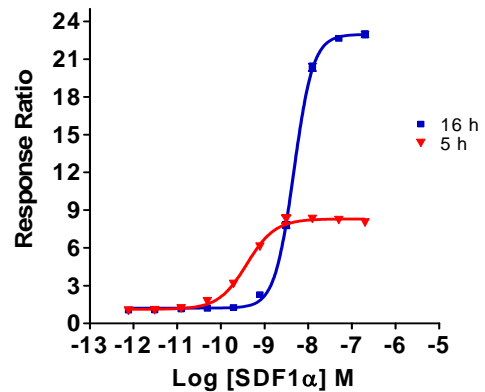
Figure 1 — Tango™ CXCR7-bla U2OS cells and Tango™ CXCR7-bla U2OS DA cells dose response to SDF1a under optimized conditions



Tango™ CXCR7-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 SDF1a (Biosource (IVGN) PHC1346) 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 SDF1a.

Assay Performance with Variable Stimulation Time

Figure 2 — Tango™ CXCR7-bla U2OS cells dose response to SDF1a with 5 or 16 hour stimulation times



Tango™ CXCR7-bla U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 16-24 hours. SDF1a (Biosource (IVGN) PHC1346) 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 SDF1a.