Tango™ CCR7-*bla* U2OS DA cells

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Catalog Numbers –K1547 and K1531

Cell Line Descriptions

Tango™ CCR7-*bla* U2OS DA (Division Arrested) cells and Tango™ CCR7-*bla* U2OS cells contain the human Chemokine (C-C motif) receptor 7 (CCR7) 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 (*bla*) reporter gene under the control of a UAS response element.

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™ CCR7-*bla* U2OS cells and the Tango™ CCR7-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of MIP-3b (Figure 1). In addition, Tango™ CCR7-*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-3β dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	21 nM	31.4 nM
Z'-factor	0.76	0.88
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 or 16 hrs
Max. [Stimulation]		= 400 nM

2. Antagonist dose response

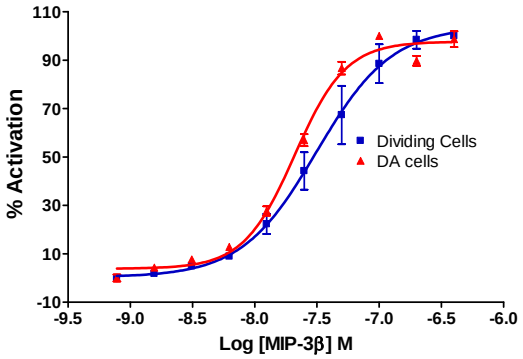
No antagonists were commercially available at the time of publication of this document.

3. Assay performance with variable stimulation time.

MIP-3β (5 Hr.) EC_{50}	= 1.6 nM
MIP-3β (16 Hr.) EC_{50}	= 31 nM

Primary Agonist Dose Response

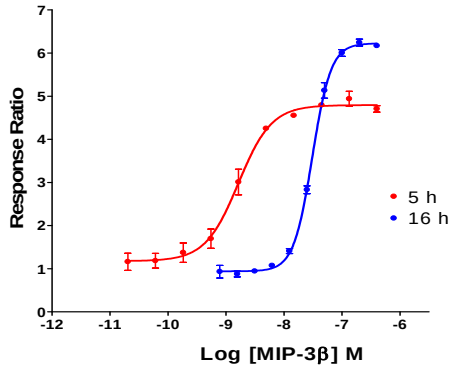
Figure 1 — Tango™ CCR7-*bla* U2OS cells and Tango™ CCR7-*bla* U2OS DA cells dose response to MIP-3β under optimized conditions



Tango™ CCR7-*bla* U2OS cells and Tango™ CCR7-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format and stimulated with MIP-3β (Sigma M3552) 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 MIP-3β.

Assay Performance with Variable Stimulation Time

Figure 2 – Tango™ CCR7-*bla* U2OS cells dose response to MIP-3β with 5 or 16 hour stimulation times



CCR7-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well plate and incubated for 16-24 hours. MIP-3β (Sigma M3552) in 0.1% DMSO was either added at the time of plating (for the 16 hour assay) or was added 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 Ratio plotted against the indicated concentrations of MIP-3β.