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**Optimization of the Tango™ CCR4-*bla* U2OS Cell Line**

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**Tango™ CCR4-*bla* U2OS cells**

Catalog Numbers – K1799

**Cell Line Descriptions**

Tango™ CCR4-*bla* U2OS cells contain the human Chemokine (C-C Motif) Receptor 4 (CCR4) 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™ CCR4-*bla* U2OS cells have been functionally validated for Z' factor and EC<sub>50</sub> concentrations of TARC (Figure 1).

## Validation Summary

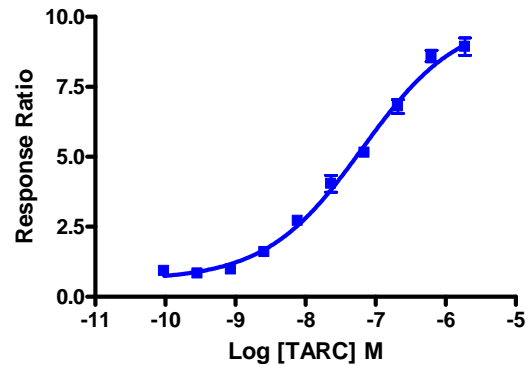
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

### 1. TARC dose response under optimized conditions

	<u>Dividing Cells</u>
EC <sub>50</sub>	65 nM
Z'-factor	0.60
Recommended cell no. /well	= 15,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 1875 nM

## Primary Agonist Dose Response

Figure 1 — Tango™ CCR4-bla U2OS cells dose response to TARC under optimized conditions



Tango™ CCR4-bla U2OS cells (15,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of TARC (Biosource PHC1264) 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 Response Ratio plotted for each replicate against the concentrations of TARC.