
Optimization of the Tango™ TACR3-*bla* U2OS Cell Line

Tango™ TACR3-*bla* U2OS DA cells**Tango™ TACR3-*bla* U2OS cells**

Catalog Numbers – K1605 and K1603

Cell Line Descriptions

Tango™ TACR3-*bla* U2OS DA (Division Arrested) cells and Tango™ TACR3-*bla* U2OS cells contain the human Tachykinin 3 (TACR3) 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. Division Arrested (DA) cells are available in an Assay Kit, which includes cells and sufficient substrate to analyze 1 x 384-well plate.

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™ TACR3-*bla* U2OS cells and the Tango™ TACR3-*bla* U2OS DA cells have been functionally validated for Z' factor and EC₅₀ concentrations of Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) (Figure 1). In addition, Tango™ TACR3-*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. Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) dose response under optimized conditions

	DA cells	Dividing Cells
EC ₅₀	747 pM	758 pM
Z'-factor	0.78	0.68
Recommended cell no. /well	= 10,000	
Recommended Stim. Time	= 5 hrs	
Max. [Stimulation]	= 312.5 nM	

2. Assay Performance with variable cell Number

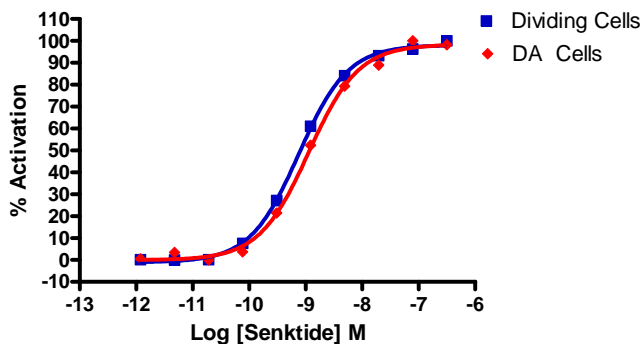
See graph below

3. Alternate agonist dose response

Neurokinin B EC₅₀ = 37.18 nM

Primary Agonist Dose Response

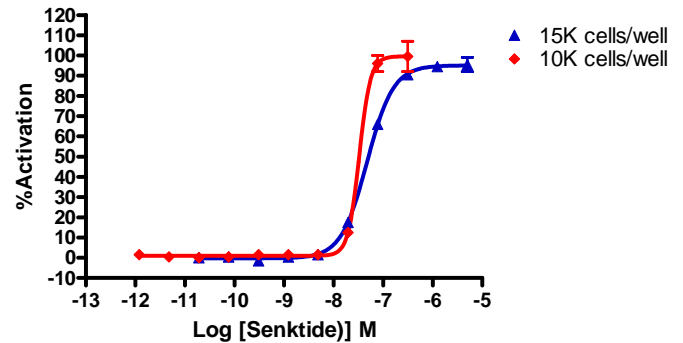
Figure 1 — Tango™ TACR3-*bla* U2OS cells and Tango™ TACR3-*bla* U2OS DA cells dose response to Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) under optimized conditions.



Tango™ TACR3-*bla* U2OS cells and Tango™ TACR3-*bla* U2OS DA 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 Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) (Sigma S6772) 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 Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide).

Assay Performance with Variable Cell Number

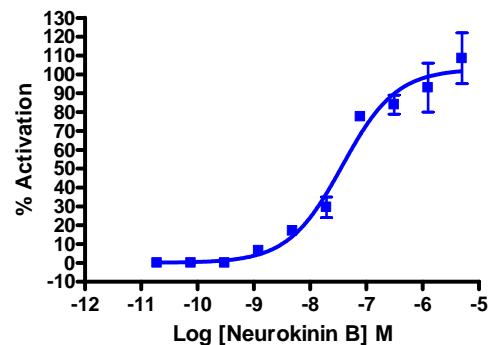
Figure 2 — Tango™ TACR3-*bla* U2OS cells dose response to Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) with 10K or 15K cells/well.



Tango™ TACR3-*bla* U2OS cells were plated in a 384-well format at 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with Succinyl-[Asp6, N-Me-Phe8]-Substance P Fragment 6-11 (Senktide) (Sigma S6772) 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of agonist.

Alternate Agonist Dose Response and Selectivity

Figure 3 — Tango™ TACR3-*bla* U2OS cells dose response to Neurokinin B.



Tango™ TACR3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Neurokinin B (Sigma #N4143) over the indicated concentration range in the presence of 0.1% DMSO for 5 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 % Activation plotted against the indicated concentrations of agonist.