
Optimization of the Tango™ SSTR1-*bla* U2OS Cell Line

Tango™ SSTR1-*bla* U2OS cells

Catalog Numbers –K1570

Cell Line Descriptions

Tango™ SSTR1-*bla* U2OS cells contain the human Somatostatin Receptor 1 (SSTR1) 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.

The Tango™ SSTR1-*bla* U2OS cells have been functionally validated for Z' factor and EC₅₀ concentrations of Somatostatin-14 (Figure 1). In addition, Tango™ SSTR1-*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. Somatostatin-14 dose response under optimized conditions

	DA cells	Dividing Cells
EC ₅₀	90 pM	229 pM
Z'-factor	0.85	0.72
Recommended cell no. /well	= 10,000	
Recommended Stim. Time	= 5 hrs	
Max. [Stimulation]	= 610 nM	

2. Assay performance with variable stimulation time.

3. Alternate agonist dose response

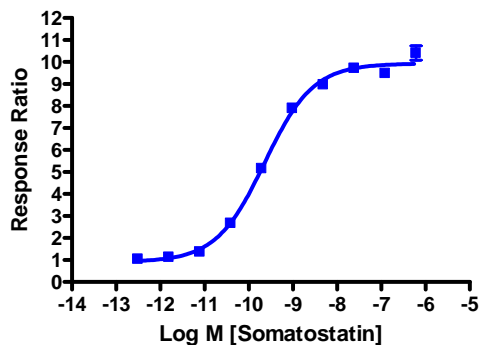
Somatostatin-28 EC₅₀ = 12.9 nM

4. Antagonist dose response

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

Primary Agonist Dose Response

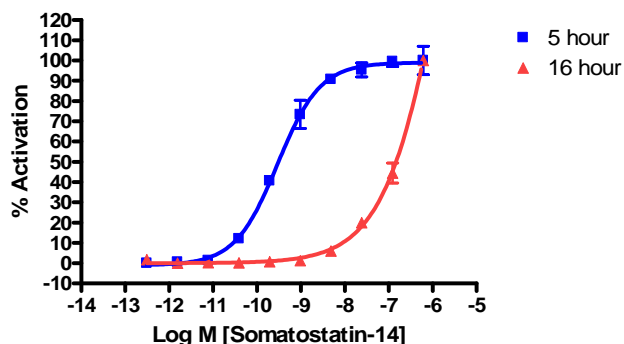
Figure 1 — Tango™ SSTR1-bla U2OS cells dose response to Somatostatin-14 under optimized conditions



Tango™ SSTR1-bla U2OS (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 Somatostatin-14 (Sigma S9129) 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 Somatostatin-14.

Assay Performance with Variable Stimulation Time

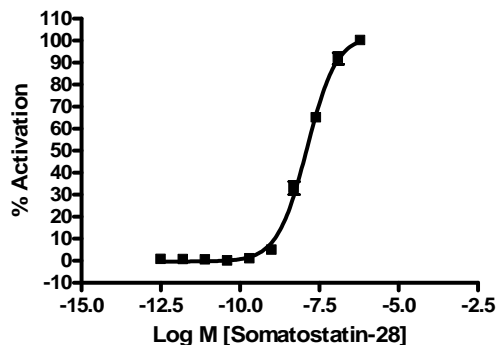
Figure 2 — Tango™ SSTR1-bla U2OS cells dose response to Somatostatin-14 with 5 and 16 hour stimulation times



Tango™ SSTR1-bla U2OS cells (10,000 cells/well) were plated the day before agonist addition in a 384-well assay plate. Somatostatin-14 (Sigma S9129) was then added to the plate over the indicated concentration range. Plates were stimulated for 5 or 16 hrs with Somatostatin-14 in 0.1% DMSO and then loaded for 2 hours with LiveBLazer™-FRET B/G Substrate. 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.

Alternate Agonist Dose Response and Selectivity

Figure 3 — Tango™ SSTR1-bla U2OS cells dose response to Somatostatin-28



Tango™ SSTR1-bla U2OS (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Somatostatin-28 (Sigma S6135) 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.