
Optimization of the Tango™ SSTR2-*bla* U2OS Cell Line

Tango™ SSTR2-*bla* U2OS DA cells**Tango™ SSTR2-*bla* U2OS cells**

Catalog Numbers – K1595 and K1582

Cell Line Descriptions

Tango™ SSTR2-*bla* U2OS DA (Division Arrested) cells and Tango™ SSTR2-*bla* U2OS cells contain the human Somatostatin Receptor 2 (SSTR2) 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™ SSTR2-*bla* U2OS cells and the Tango™ SSTR2-*bla* U2OS DA cells have been functionally validated for Z' factor and EC₅₀ concentrations of Somatostatin-14 (Figure 1). In addition, Tango™ SSTR2-*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	169 pM	86 pM
Z'-factor	0.92	0.95
Recommended cell no. /well	= 10,000	= 10,000
Recommended Stim. Time	= 5 hrs	= 5 hrs
Max. [Stimulation]	= 610 nM	= 610 nM

2. Alternate agonist dose response

Somatostatin-28 EC ₅₀	= 7.6 nM
Seglitide EC ₅₀	= 91.2 pM

3. Antagonist dose response

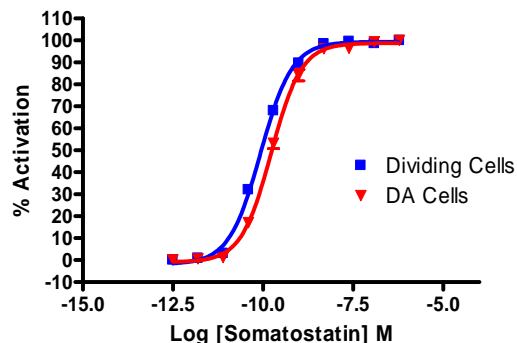
CYN154806 Dividing IC ₅₀	= 10 nM
CYN154806 Cryo IC ₅₀	= 31 nM
CYN154806 DA IC ₅₀	= 11 nM

Assay Testing Summary

4. Assay performance with variable stimulation time.

Primary Agonist Dose Response

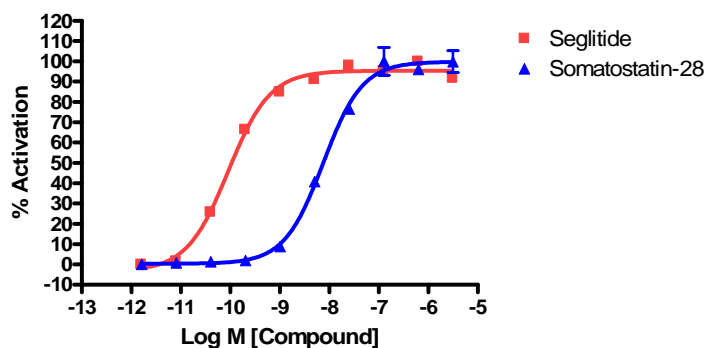
Figure 1 — Tango™ SSTR2-bla U2OS cells and Tango™ SSTR2-bla U2OS DA cells dose response to Somatostatin-14 under optimized conditions



Tango™ SSTR2-bla U2OS cells and Tango™ SSTR2-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 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. 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

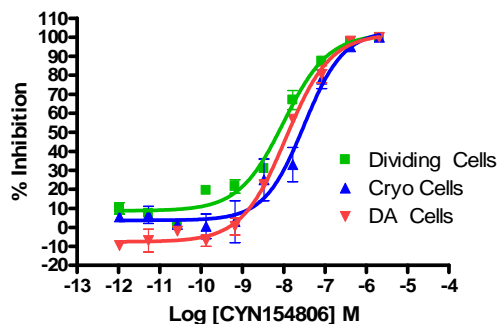
Figure 2 — Tango™ SSTR2-bla U2OS cells dose response to Somatostatin-28 and Seglitide under optimized conditions



Tango™ SSTR2-bla U2OS cells (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) and Seglitide (Sigma S1316) 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.

Antagonist Dose Response

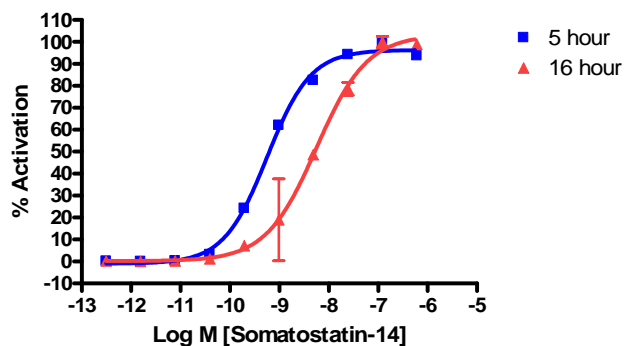
Figure 3 — Tango™ SSTR2-*bla* U2OS cells dose response to CYN154806



Tango™ SSTR2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to CYN154806 (Sigma CN2490) for 30 min. and then stimulated with an EC80 concentration 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of CYN154806.

Assay Performance with Variable Stimulation Time

Figure 4 — Tango™ SSTR2-*bla* U2OS cells dose response to Somatostatin-14 with 5 and 16 hour stimulation times



Tango™ SSTR2-*bla* U2OS cells (10,000 cells/well) were plated the day before agonist addition in a 384-well assay plate. Somatostatin-14 (Sigma S9129) 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 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.