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**Optimization of the Tango™ F2RL1-*bla* U2OS Cell Line**

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**Tango™ F2RL1-*bla* U2OS cells**

Catalog Numbers – K1830

**Cell Line Descriptions**

Tango™ F2RL1-*bla* U2OS DA (Division Arrested) cells and Tango™ F2RL1-*bla* U2OS cells contain the human Coagulation factor II (thrombin) receptor-like 1 (F2RL1) 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™ F2RL1-*bla* U2OS cells have been functionally validated for Z' factor and EC<sub>50</sub> concentrations of SLIGRL (Figure 1).

## Validation Summary

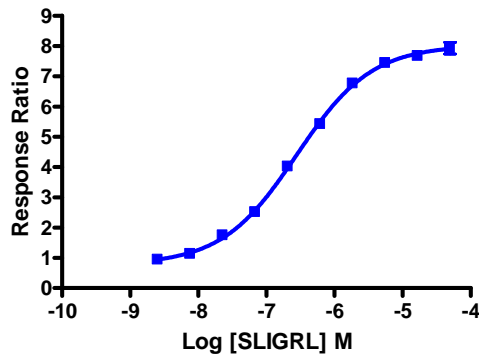
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

### 1. SLIGRL dose response under optimized conditions

	<u>Dividing Cells</u>
EC <sub>50</sub>	270nM
Z'-factor	0.73
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 50000 nM

## Primary Agonist Dose Response

Figure 1 — Tango™ F2RL1-*bla* U2OS cells and Tango™ F2RL1-*bla* U2OS DA cells dose response to SLIGRL under optimized conditions



Tango™ F2RL1-*bla* U2OS cells and Tango™ F2RL1-*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 SLIGRL (Sigma S9317) 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 Response Ratio plotted for each replicate against the concentrations of SLIGRL.