
Optimization of the Tango™ EDG3-*bla* U2OS Cell Line

Tango™ EDG3-*bla* U2OS cells

Catalog Numbers – Early Access,

Cell Line Descriptions

Tango™ EDG3-*bla* U2OS cells contain the human Endothelial Differentiation Gene 3 (EDG3) 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™ EDG3-*bla* U2OS cells have been functionally validated for Z' factor and EC₅₀ concentrations of S1P (Figure 1). In addition, Tango™ EDG3-*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. S1P dose response under optimized conditions

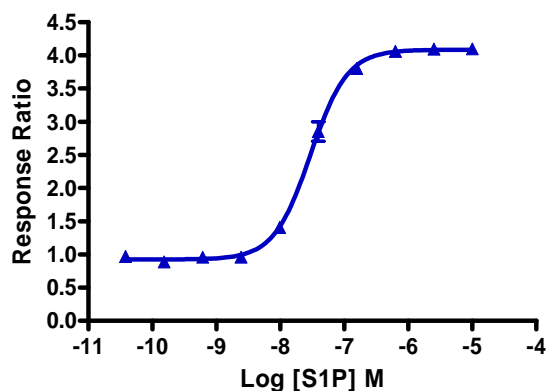
	<u>Dividing Cells</u>
EC ₅₀	29 nM
Z'-factor	0.80
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 10000 nM

Assay Testing Summary

2. Assay performance in 2nd messenger assay.

Primary Agonist Dose Response

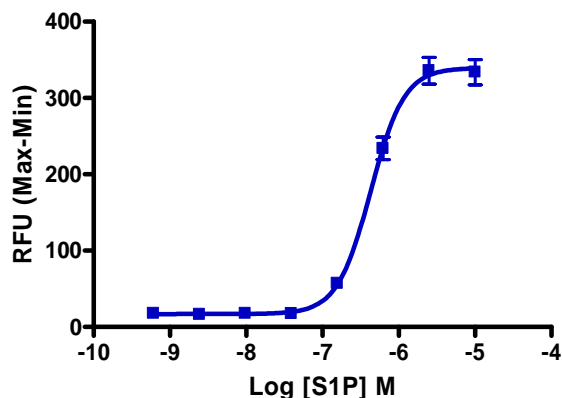
Figure 1 — Tango™ EDG3-*bla* U2OS cells dose response to S1P under optimized conditions



Tango™ EDG3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 48 hours. Cells were stimulated with a dilution series of S1P (Avanti Polar Lipids 860492P) 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 S1P.

2nd Messenger Dose Response

Figure 2 — Tango™ EDG3-*bla* U2OS 2nd messenger dose response to S1P under optimized conditions.



Tango™ EDG3-*bla* U2OS cells were loaded with Fluo4 NW and tested for a response to S1P.