Tango™ EDG7-*bla* U2OS cells

Catalog Numbers – K1849

Cell Line Descriptions

Tango™ EDG7-*bla* U2OS cells contain the human Endothelial Differentiation Gene 7 (EDG7) 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™ EDG7-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of LPA (Figure 1). In addition, Tango™ EDG7-*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**

	Dividing Cells
EC_{50}	1.2 µM
Z'-factor	0.75
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 100,000 nM

2. **Alternate agonist dose response**

VPC31143(R) EC50 = 199 nM

3. **Antagonist dose response**

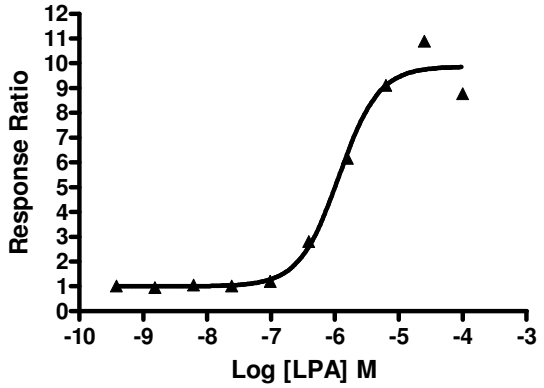
VPC32183 IC50 = 346 nM

Assay Testing Summary

4. **Assay performance in 2nd messenger assay.**

Primary Agonist Dose Response

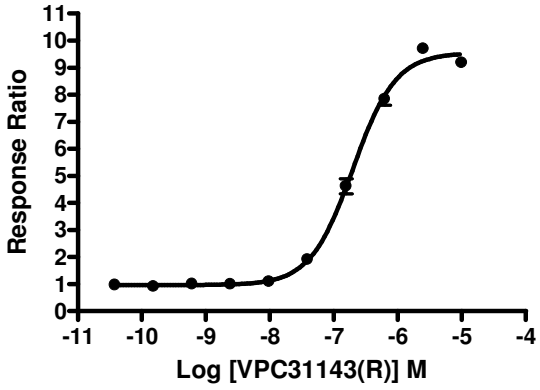
Figure 1 — Tango™ EDG7-*bla* U2OS cells dose response to LPA under optimized conditions



Tango™ EDG7-*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 LPA (Avanti Polar Lipids 857130P) 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 the Response Ratio is plotted for each replicate against the concentrations of LPA.

Alternate Agonist Dose Response and Selectivity

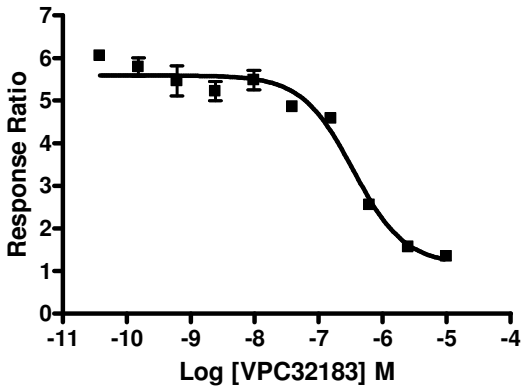
Figure 2 — Tango™ EDG7-bla U2OS cells dose response to VPC31143(R).



Tango™ EDG7-bla U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 48 hours prior to stimulation with VPC31143(R) (Avanti Polar Lipids 857353P) 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 Response Ratio is plotted against the indicated concentrations of agonist.

Antagonist Dose Response

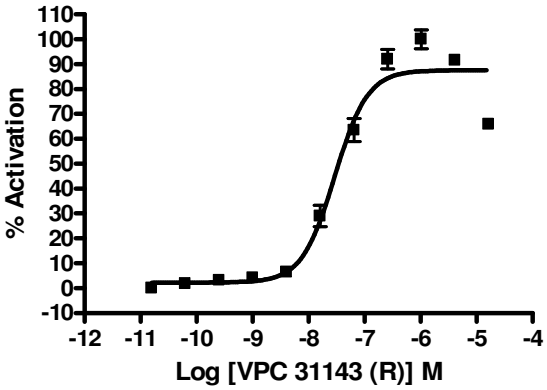
Figure 3 — Tango™ EDG7-bla U2OS cells dose response to VPC32183



Tango™ EDG7-bla U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 48 hours. Cells were exposed to VPC32183 (Avanti Polar Lipids 857340P) for 30 min. and then stimulated with an EC80 concentration of LPA (Avanti Polar Lipids 857130P) 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 the Response Ratio is plotted against the indicated concentrations of VPC32183.

2nd Messenger Dose Response

Figure 2 — Tango™ EDG7-*bla* U2OS 2nd messenger dose response to VPC31143(R) under optimized conditions.



Tango™ EDG7-*bla* U2OS cells were loaded with Fluo4 and tested for a response to VPC31143(R).