
Optimization of the Tango™ EDG6-*bla* U2OS Cell Line

Tango™ EDG6-*bla* U2OS DA cells**Tango™ EDG6-*bla* U2OS cells**

Catalog Numbers – K1757 and K1622

Cell Line Descriptions

Tango™ EDG6-*bla* U2OS DA (Division Arrested) cells and Tango™ EDG6-*bla* U2OS cells contain the human Endothelial Differentiation Gene 6 (EDG6) 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. Division Arrested (DA) cells are available as an Assay Kit, which includes cells and sufficient substrate to analyze 1 x 384-well plate.

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™ EDG6-*bla* U2OS cells and the Tango™ EDG6-*bla* U2OS DA cells have been functionally validated for Z' factor and EC₅₀ concentrations of Sphingosine-1-phosphate (Figure 1). In addition, Tango™ EDG6-*bla* U2OS cells have been tested for assay performance under variable conditions.

Target Description

S1P is a bioactive lysophospholipid with diverse biological functions. It is a key cell signaling molecule that has been shown to act as both an intracellular second messenger and an extracellular ligand for a related group of 5 GPCRs in the endothelial differentiation gene (EDG) family of receptors (1,2). S1P is a polar sphingolipid metabolite that is derived through the multi-step enzymatic metabolism of the abundant membrane phospholipid, sphingomyelin (3). S1P is found at high nM levels in human serum and plasma where it is bound extensively by albumin and other plasma proteins (3,4). Most of the S1P in the blood is released from activated platelets (5), while a small percentage of circulating S1P is released by other blood cells (6).

The S1P receptors were originally classified in the EDG family of receptors. The EDG family was later divided into two distinct groups of receptors based on their ligand specificity for either Lysophosphatidic acid (LPA) or S1P. There are three high affinity LPA receptors LPA₁, LPA₂, and LPA₃ (formally EDG2, EDG4, and EDG7), and five high affinity S1P receptors S1P₁, S1P₂, S1P₃, S1P₄ and S1P₅ (formally EDG1, EDG5, EDG3, EDG6, and EDG8). S1P₁₋₅ are differentially linked via G_i, G_q, G_{12/13}, and Rho to multiple effector systems and diverse biological functions (3).

Validation Summary

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

1. Sphingosine-1-phosphate (S1P) dose response under optimized conditions

	DA cells	Dividing Cells
EC ₅₀	5nM	17nM
Z'-factor	0.71	0.73

Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 10000 nM

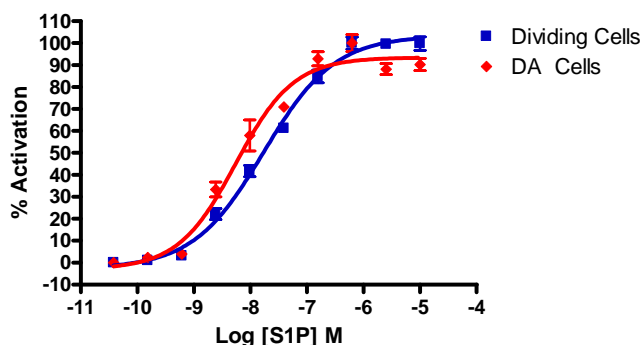
2. Alternate agonist dose response

dhS1P = 26 nM

3. Assay performance with variable cell number.

Primary Agonist Dose Response

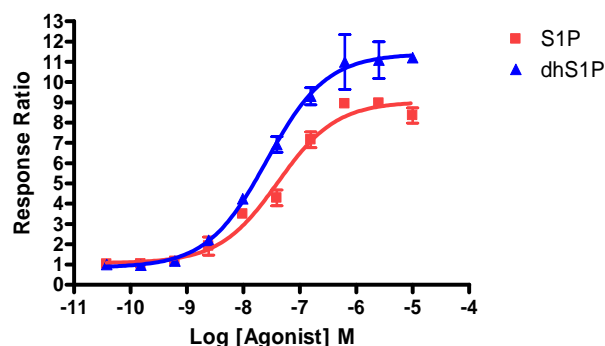
Figure 1 — Tango™ EDG6-*bla* U2OS cells dose response to S1P under optimized conditions



Tango™ EDG6-*bla* U2OS cells and Tango™ EDG6-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 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 % Activation plotted for each replicate against the concentrations of S1P.

Alternate Agonist Dose Response and Selectivity

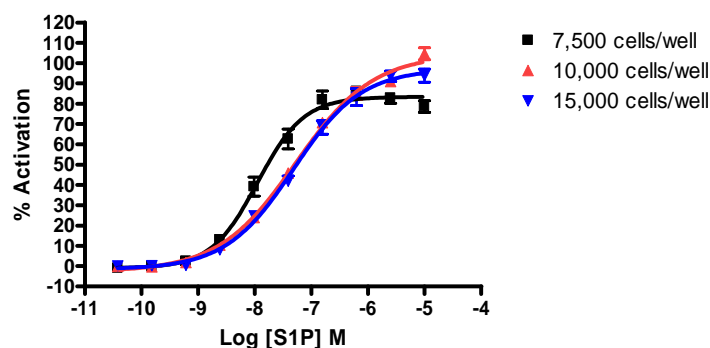
Figure 2 — Tango™ EDG6-*bla* U2OS cells dose response to S1P and dhS1P



Tango™ EDG6-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with CP Sphingosine-1-phosphate (Avanti Polar Lipids 860492P) and Dihydrospingosine-1-phosphate (Avanti Polar Lipids 860536P) 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.

Assay Performance with Variable Cell Number

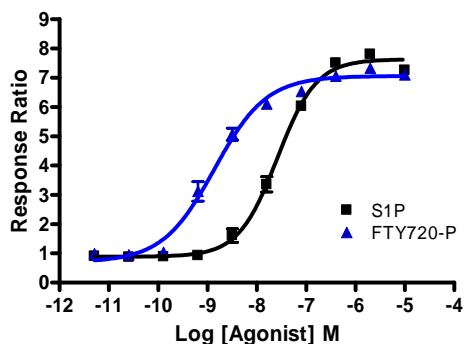
Figure 5 — Tango™ EDG6-*bla* U2OS cells dose response to Sphingosine-1-phosphate with 7.5K 10K or 15K cells/well



Tango™ EDG6-*bla* U2OS cells were plated in a 384-well format at 7,500 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with Sphingosine-1-phosphate (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 the Response Ratios plotted against the indicated concentrations of Sphingosine-1-phosphate.

Alternate Agonist Dose Response and Selectivity

Figure 2 — Tango™ EDG6-*bla* U2OS cells dose response to FTY720-P



Tango™ EDG6-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 48 hours prior to stimulation with FTY720-P (Toronto Research Chemicals F805005) or S1P 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 plotted against the indicated concentrations of agonist.

References

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- 4) Rosen, H., Goetzl, E. (2005) *Nature Reviews Immunology* **5**, 560-570
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