Tango™ EDG8-*bla* U2OS cells

Catalog Numbers – K1518

Cell Line Descriptions

Tango™ EDG8-*bla* U2OS cells contain the human Endothelial Differentiation Gene 8 (EDG8) 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.

The Tango™ EDG8-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of Sphingosine-1-phosphate (Figure 1). In addition, Tango™ EDG8-*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 LPA_1, LPA_2, and LPA_3 (formally EDG2, EDG4, and EDG7), and five high affinity S1P receptors $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$ (formally EDG1, EDG5, EDG3, EDG6, and EDG8). $S1P_{1-5}$ are differentially linked via G_i, G_q, $G_{12/13}$, and Rho to multiple effector systems and diverse biological functions (3).

Validation Summary

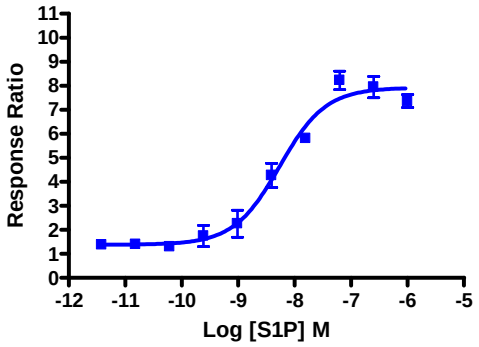
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Sphingosine-1-phosphate dose response under optimized conditions**

	Dividing Cells
EC_{50}	5.4 nM
Z'-factor	0.65
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 1000 nM

Primary Agonist Dose Response

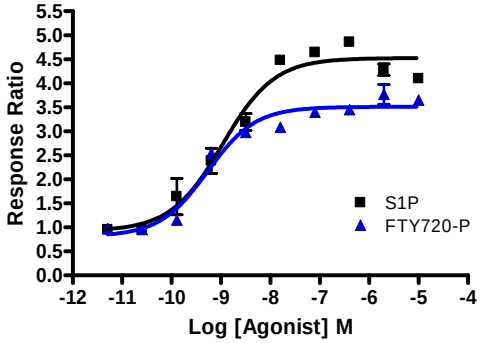
Figure 1 — Tango™ EDG8-*bla* U2OS cells dose response to Sphingosine-1-phosphate under optimized conditions



Tango™ EDG8-*bla* U2OS 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 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 % Activation plotted for each replicate against the concentrations of Sphingosine-1-phosphate.

Alternate Agonist Dose Response and Selectivity

Figure 2 — Tango™ EDG8-*bla* U2OS cells dose response to FTY720-P



Tango™ EDG8-*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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