
Optimization of the Tango™ EDG2-*bla* U2OS Cell Line

Tango™ EDG2-*bla* U2OS cells

Catalog Numbers –K1519

Cell Line Descriptions

Tango™ EDG2-*bla* U2OS cells contain the human Endothelial Differentiation Gene 2 (EDG2) 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™ EDG2-*bla* U2OS cells have been functionally validated for Z' factor and EC₅₀ concentrations of LPA (18:1) (Figure 1). In addition, Tango™ EDG2-*bla* U2OS cells have been tested for assay performance under variable conditions.

Target Description

LPA is a simple naturally occurring bioactive lipid that has diverse effects on several cell types. LPA induces proliferative and/or morphological effects and has been proposed to be involved in biologically important processes including neurogenesis, myelination, angiogenesis, wound healing, and cancer progression (1). All cells contain small amounts of LPA associated with membrane biosynthesis (1). In addition, serum contains LPA at micro molar concentrations (2).

The LPA 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 Sphingosine-1-Phosphate (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). The LPA are differentially linked via G_i and G_q to multiple effector systems and diverse biological functions (1).

Validation Summary

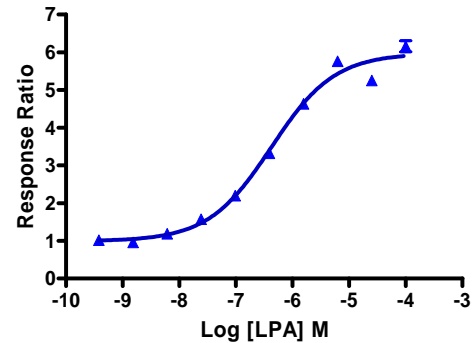
Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. LPA (18:1) dose response under optimized conditions

	<u>Dividing Cells</u>
EC ₅₀	380 nM
Z'-factor	0.71
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 100000 nM

Primary Agonist Dose Response

Figure 1 — Tango™ EDG2-*bla* U2OS cells dose response to LPA (18:1) under optimized conditions



Tango™ EDG2-*bla* U2OS 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 LPA (18:1) (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 plotted for each replicate against the concentrations of LPA (18:1).

References

- 1) Contos, J.A., Ishii, I., and Chun, J. (2000) Mol Pharmacol **58**, 1188-1196
- 2) Noguchi, K., Ishii, S., and Shimizu, T. (2003) J Biol Chem. **278**, 25600-6