
Optimization of the Tango™ GPR92-*bla* U2OS Cell Line

Tango™ GPR92-*bla* U2OS cells

Catalog Numbers – Early Access

Cell Line Descriptions

Tango™ GPR92-*bla* U2OS cells contain the human G protein-coupled receptor 92 (GPR92) 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™ GPR92-*bla* U2OS cells have been functionally validated for Z' factor and a functional response to FBS (Figure 2). In addition, Tango™ GPR92-*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. LPA dose response under optimized conditions

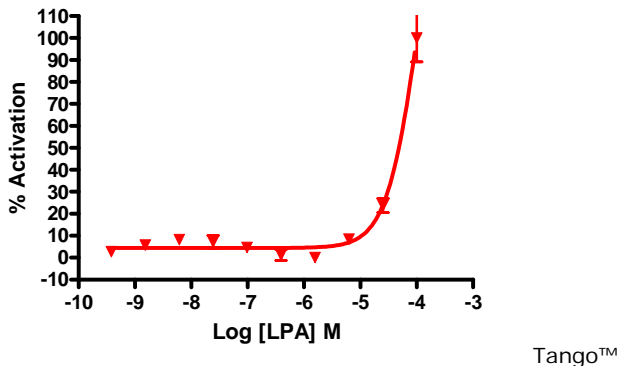
	<u>Dividing Cells</u>
EC ₅₀	= >10,000 nM
Z'-factor	= 0.5
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 48 hrs
Max. [Stimulation]	= 100,000 nM

2. Alternate agonist dose response

3. Assay performance with variable stimulation time.

Primary Agonist Dose Response

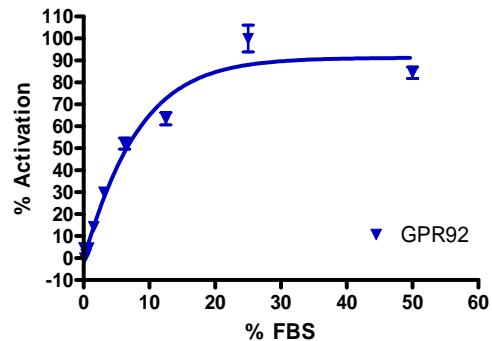
Figure 1 — Tango™ GPR92-*bla* U2OS cells dose response to LPA under optimized conditions



GPR92-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 24 hours. Cells were stimulated with a dilution series of LPA (Avanti Polar Lipids 857130P) in the presence of 0.1% DMSO for 48 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 LPA.

FBS Dose Response

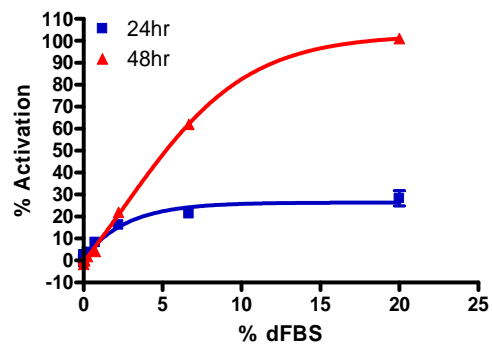
Figure 2 — Tango™ GPR92-*bla* U2OS cells dose response to FBS



Tango™ GPR92-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 24 hours prior to stimulation with dFBS (Invitrogen 26400) over the indicated concentration range in the presence of 0.1% DMSO for 48 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 Stimulation Time

Figure 3 — Tango™ GPR92-*bla* U2OS cells dose response to FBS with 24 or 48 hour stimulation times



Tango™ GPR92-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well assay plate and incubated 24 hours. FBS (Invitrogen 26400) was added for 24 or 48 hours. The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence 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 dFBS.