Tango™ PTAFR-*bla* U2OS cells

Catalog Numbers –K1789

Cell Line Descriptions

Tango™ PTAFR-*bla* U2OS cells contain the human Platelet Activating Factor Receptor (PTAFR) 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™ PTAFR-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine (Figure 1). In addition, Tango™ PTAFR-*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. β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine dose response under optimized conditions

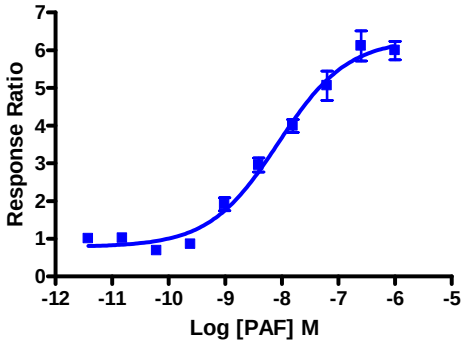
	Dividing Cells
EC_{50}	1.3 nM
Z'-factor	0.80
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 600 nM

2. Antagonist dose response

ABT-491 IC_{50}= 1.8 nM Dividing cells
ABT-491 IC_{50}= 1.2 nM DA cells

Primary Agonist Dose Response

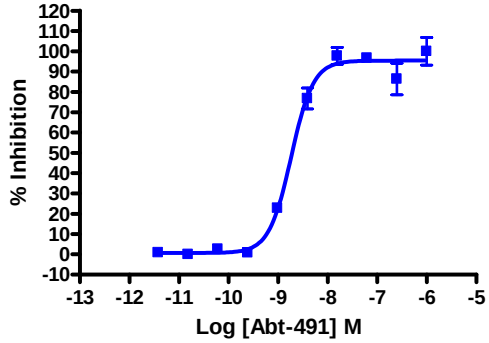
Figure 1 — Tango™ PTAFR-*bla* U2OS cells dose response to β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine under optimized conditions



Tango™ PTAFR-*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 β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine (Sigma P4904) 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 Response Ratio plotted for each replicate against the concentrations of β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine.

Antagonist Dose Response

Figure 3 — Tango™ PTAFR-*bla* U2OS cells dose response to ABT-491



Tango™ PTAFR-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to ABT-491 (Sigma A9227) for 30 min. and then stimulated with an EC_{80} concentration of β-Acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine (Sigma P4904) 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of ABT-491.