

## T-REx™ Tango™ GPR37-*bla* U2OS cells

Catalog Numbers – Early Access

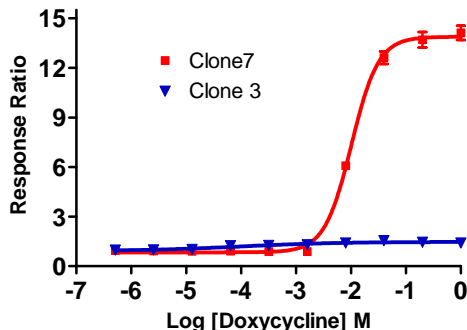
### Cell Line Descriptions

T-REx™ Tango™ GPR37-*bla* U2OS cells contain the orphan human G-protein Coupled Receptor 37 (GPR37) linked to a TEV protease site and a Gal4-VP16 transcription factor stably integrated into the Tango™ GPCR-*bla* U2OS parental cell line. Expression of GPR37 is controlled by the T-REx™ system. 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 T-REx™ Tango™ GPR37-*bla* U2OS cells have been functionally validated for a receptor specific response to FBS. (Figure 1). In addition, T-REx™ Tango™ GPR37-*bla* U2OS cells have been tested for assay performance under variable conditions.

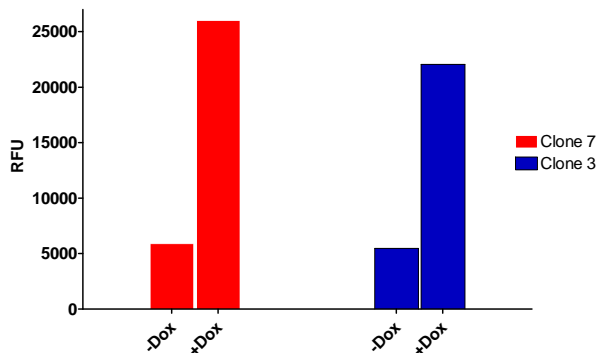
## Validation Summary

**Figure 1 — T-REx™ Tango™ GPR37-*bla* U2OS cells response to FBS under optimized conditions**



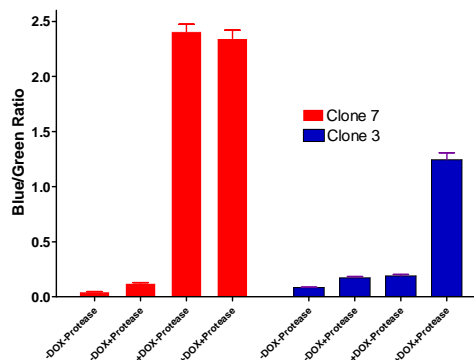
TREx™ Tango™ GPR37-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format, induced with varying concentrations of doxycycline in DMEM containing 1% dFBS, and incubated for 16-20 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values 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 Doxycycline.

**Figure 2 — GPR37 expression in T-REx™ Tango™ GPR37-*bla* U2OS cells.**



TREx™ Tango™ GPR37-*bla* U2OS cells (clone 7 and clone 3) were stained using anti-VP16 antibody (abcam ab4808). When induced with 500 nM doxycycline for 24 h the TREx™ Tango™ GPR37-*bla* U2OS cells showed significantly higher expression of GPR37, as detected by anti-VP16 antibody staining.

**Figure 3 — Receptor specific response to protease overexpression in T-REx™ Tango™ GPR37-*bla* U2OS cells.**



TREx™ Tango™ GPR37-*bla* U2OS cells (30,000 cells/well) were plated in a 96-well format, transfected with TEV-protease or a control vector using Lipofectamine2000™. After 24 h of transfection, the cells were induced with doxycycline and incubated for 16-20 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 460/530 ratios plotted for each replicate.

NOTE: As evident in figures 1, 2 and 3, TREx™ Tango™ GPR37-*bla* U2OS, clones 7 and 3 both express the receptor when induced with doxycycline, but is constitutively active only in case of clone 7. In clone 3 the receptor is not constitutively active but is functional/cleavable as shown in figure 3.