
Optimization of the Tango™ GALR1-*bla* U2OS Cell Line

Tango™ GALR1-*bla* U2OS DA cells**Tango™ GALR1-*bla* U2OS cells**

Catalog Numbers – K1617 and K1616

Cell Line Descriptions

Tango™ GALR1-*bla* U2OS DA (Division Arrested) cells and Tango™ GALR1-*bla* U2OS cells contain the human Galanin Receptor 1 (GALR1) 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.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both the Tango™ GALR1-*bla* U2OS cells and the Tango™ GALR1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC₅₀ concentrations of Galanin (Figure 1). In addition, Tango™ GALR1-*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. Galanin dose response under optimized conditions

	DA cells	Dividing Cells
EC ₅₀	108 nM	128.2 nM
Z'-factor	0.86	0.92
Recommended cell no. /well	= 10,000	= 10,000
Recommended Stim. Time	= 16 hrs	= 16 hrs
Max. [Stimulation]	= 10,000 nM	= 10,000 nM

2. Alternate agonist dose response

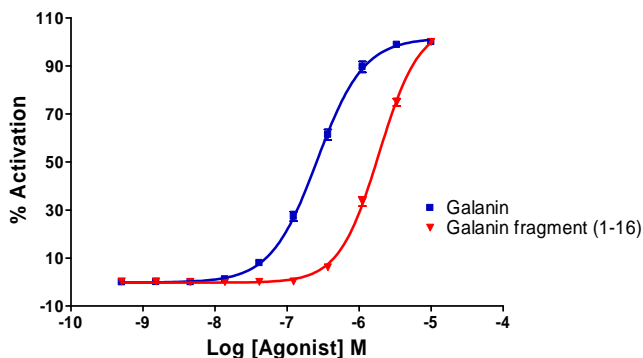
Galanin fragment (1-16) EC₅₀ = 1.89 μM

3. Assay performance with variable stimulation time.

Galanin(5 Hr.) EC₅₀ = 394.1 nM
 Galanin (16 Hr.) EC₅₀ = 168.8 nM

Alternate Agonist Dose Response and Selectivity

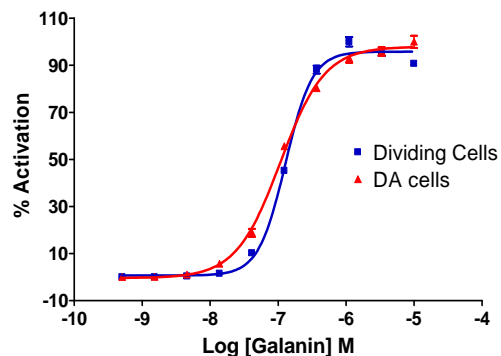
Figure 2 — Tango™ GALR1-bla U2OS cells dose response to Galanin and Galanin fragment (1-16).



Tango™ GALR1-bla U2OS cells (10,000 cells/well) were plated in a 384-well format and stimulated with Galanin (Sigma G0278) or Galanin fragment (1-16) (Sigma, G112) over the indicated concentration range in the presence of 0.1% DMSO for 16 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. The data shows the correct rank order potency.

Primary Agonist Dose Response

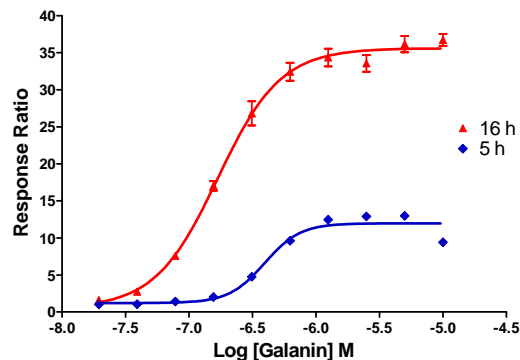
Figure 1 — Tango™ GALR1-bla U2OS cells and Tango™ GALR1-bla U2OS DA cells dose response to Galanin under optimized conditions



Tango™ GALR1-bla U2OS cells and Tango™ GALR1-bla U2OS DA cells (10,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of Galanin (Sigma G0278) in the presence of 0.1% DMSO for 16 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 Galanin.

Assay Performance with Variable Stimulation Time

Figure 3 — Tango™ GALR1-bla U2OS cells dose response to Galanin with 5 or 16 hour stimulation times



GALR1-bla U2OS cells (10,000 cells/well) were plated in a 384-well plate and incubated for 16-24 hours. Galanin (Sigma G0278) in 0.1% DMSO was either added at the time of plating (for the 16 hour assay) or was added for 5 hours after the overnight incubation (for the 5 hour assay). 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 Response Ratios plotted against the indicated concentrations of Galanin.