Tango™ GLP2R-*bla* U2OS cells

Catalog Numbers – K1543

Cell Line Descriptions

Tango™ GLP2R-*bla* U2OS cells contain the human Glucagon-like Peptide 2 Receptor (GLP2R) 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™ GLP2R-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of Glucagon-like peptide 2 arg (Figure 1). In addition, Tango™ GLP2R-*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. **Glucagon-like peptide 2 arg dose response under optimized conditions**

	Dividing Cells
EC_{50}	38.09 nM
Z'-factor	0.81
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 20000 nM

2. **Assay performance with variable stimulation time.**

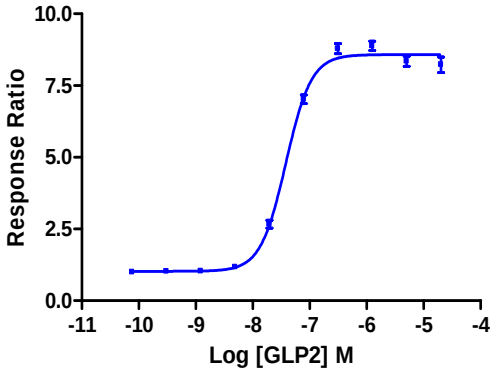
GLP2 (5 Hr.) EC_{50}	= 13 nM
GLP2 (16 Hr.) EC_{50}	= 47 nM

3. **Agonist 2nd messenger dose response**

GLP2 EC_{50} = 0.307 nM

Primary Agonist Dose Response

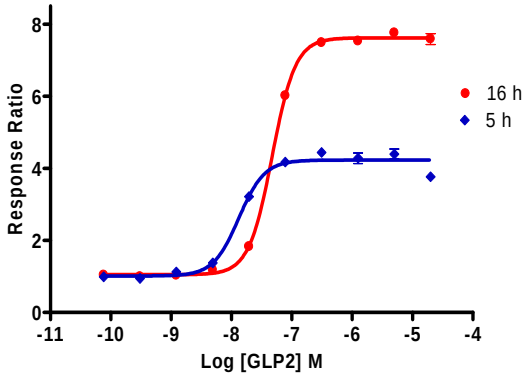
Figure 1 — Tango™ GLP2R-*bla* U2OS cells dose response to Glucagon-like peptide 2 arg under optimized conditions



Tango™ GLP2R-*bla* U2OS cells and Tango™ GLP2R-*bla* U2OS DA cells (10,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of Glucagon-like peptide 2 arg (Sigma G8166) 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 Response Ratio plotted for each replicate against the concentrations of Glucagon-like peptide 2 arg.

Assay Performance with Variable Stimulation Time

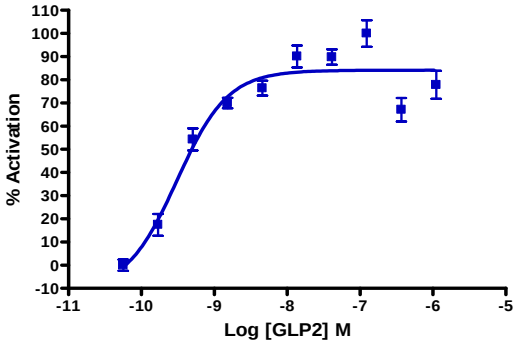
Figure 2 – Tango™ GLP2R-*bla* U2OS cells dose response to Glucagon-like peptide 2 arg with 5 or 16 hour stimulation times



Tango™ GLP2R-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well plate and incubated for 16-24 hours. Glucagon-like peptide 2 arg (Sigma G8166) 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 ratio plotted against the indicated concentrations of Glucagon-like peptide 2 arg.

2nd Messenger Dose Response

Figure 3 — Tango™ GLP2R-*bla* U2OS 2nd messenger dose response to GLP2 under optimized conditions.



Tango™ GLP2R-*bla* U2OS cells were tested for a response to GLP2 with a TR-FRET cAMP kit.