Tango™ EDNRA-*bla* U2OS cells

Catalog Numbers – K1444

Cell Line Descriptions

Tango™ EDNRA-*bla* U2OS cells contain the human Endothelin Receptor A (EDNRA) 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™ EDNRA-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of Endothelin-1 (Figure 1). In addition, Tango™ EDNRA-*bla* U2OS cells have been tested for assay performance under variable conditions.

Target Description

The endothelin (ET) axis is primarily known for its involvement in the regulation of blood pressure and maintenance of vasculature tone (1). Endothelins stimulate vasoconstriction through EDNRA signaling in smooth muscle cells of blood vessels (1). In fact, ET-1 is the most potent and sustained vasoconstrictor yet to be identified (2). Endothelins can also stimulate vasodilatation through EDNRB signaling in endothelial cells (1). The endothelin axis plays a role in many physiological and cellular responses. These include but are not limited to, connective tissue remodeling (3), ovarian and urologic tumor growth and progression (4, 5), chronic heart failure (6), contraction of airway and intestinal smooth muscle (7, 8), solid organ transplant and vein graft failure (9, 10), stimulation of natriuretic peptide release from atria (11), inhibition of renin release from rena glomeruli (11), and the development of neural crest cells in the embryo (1).

The endothelin axis consists of three 21 amino acid peptides that are designated ET-1, ET-2, and ET-3, and two G-protein coupled receptors EDNRA and EDNRB (1). The endothelin peptides 1, 2, and 3 are produced by the proteolysis of larger 38 amino acid precursors known as big endothelins (11). EDNRA shows a high affinity for ET-1 and ET-2 but not ET-3, whereas EDNRB shows high affinity for all three peptides (1).

Endothelin receptors are expressed in all tissues (12, 13). EDNRA is expressed at high levels in the smooth muscle of blood vessels whereas EDNRB is expressed at a lower level in this tissue (12). EDNRB has been shown to be expressed at high levels in the kidney where it may act as a "clearing receptor" for blood born endothelin (12). EDNRB is also localized to the endothelial cells that line all blood vessels (12). For a recent review of the endothelin axis see (13).

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. Endothelin-1 dose response under optimized conditions

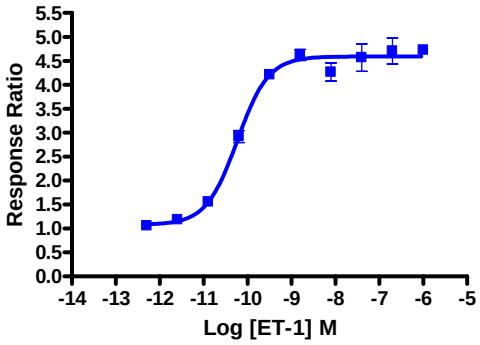
	Dividing Cells
EC_{50}	60 pM
Z'-factor	0.87
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 1,000 nM

2. Antagonist dose response

PD142893 IC_{50}= 16 nM

Primary Agonist Dose Response

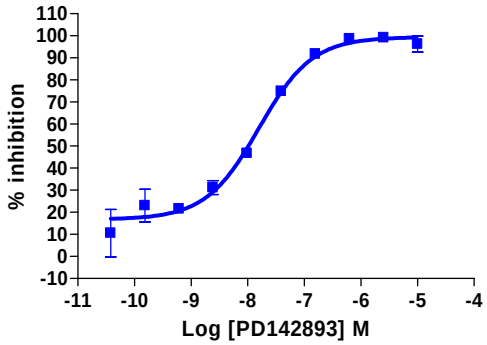
Figure 1 — Tango™ EDNRA-*bla* U2OS cells and Tango™ EDNRA-*bla* U2OS DA cells dose response to Endothelin-1 under optimized conditions



Tango™ EDNRA-*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 Endothelin-1 (Calbiochem 05-23-3800) 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 % Activation plotted for each replicate against the concentrations of Endothelin-1.

Antagonist Dose Response

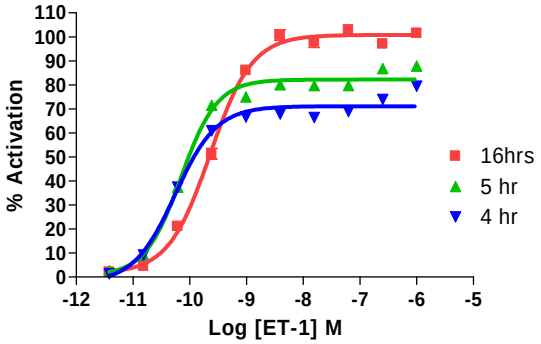
Figure 3 — Tango™ EDNRA-*bla* U2OS cells dose response to PD142893



Tango™ EDNRA-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to PD142893 (Sigma P-2959) for 30 min. and then stimulated with an EC80 concentration of Endothelin-1 (Calbiochem 05-23-3800) 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 PD142893.

Assay Performance with Variable Stimulation Time

Figure 3 – Tango™ EDNRA-*bla* U2OS cells dose response to Endothelin-1 with 5 or 16 hour stimulation times



Tango™ EDNRA-*bla* U2OS cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. Endothelin-1 (Calbiochem 05-23-3800) was then added to the plate over the indicated concentration range for 4, 5 or 16 hrs in 0.1% DMSO. 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 Endothelin-1.

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