GeneBLAzer® EDNRB HEK 293T DA Assay Kit

GeneBLAzer® EDNRB NFAT-*bla* HEK 293T Cells

Catalog Numbers – K1349 and K1727

Cell Line Descriptions

GeneBLAzer® EDNRB HEK 293T DA (Division Arrested) cells and GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells contain the human Endothelin type B (EDNRB) receptor (Accession# NM_000115) stably integrated into the CellSensor® NFAT-*bla* HEK 293T cell line. CellSensor® NFAT-*bla* HEK 293T cells (Cat. no. K1538) contain a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells(NFAT) response element. Division Arrested (DA) cells are available as Assay Kit,which includes cells and sufficient substrate to analyze 1 x 384-well plate.

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 GeneBLAzer® EDNRB HEK 293T DA cells and GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells are functionally validated for Z'-factor and EC_{50} concentrations of ET-1, (Figure 1). In addition, GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also included.

Target Description

The Endothelin (ET) axis is primarily known for its involvement in the regulation of blood pressure and maintenance of vasculature tone (1). The 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). The 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 of the the peptides (1).

The 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).

Validation Summary

Performance of this assay was evaluated under various conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **ET-1 agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	313 pM	217 pM
Z'-factor	0.77	0.80

Recommended cell no. = 5K cells/well
Recommended [DMSO] = up to 0.5%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 0.1µM

2. **Alternate agonist dose response**
   ET-2 EC_{50} = 1.1 nM
   ET-3 EC_{50} = 216 pM

3. **Antagonist dose response**
   See antagonist dose response section

4. **Agonist dose response using Fluo-4NW**
   ET-1 EC_{50} = 640pM

Assay Development Summary

5. **Assay Performance with variable cell number**

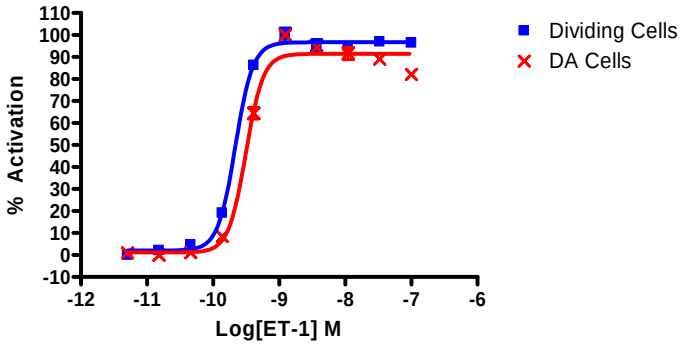
6. **Assay Performance with variable Stimulation Time**

7. **Assay Performance with variable Substrate loading time**

8. **Assay Performance with variable DMSO concentrations**

Primary Agonist Dose Response

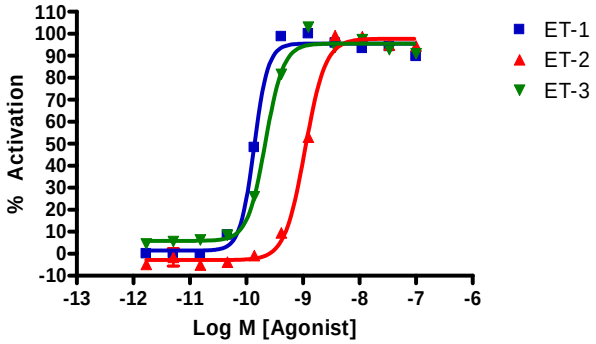
Figure 1 — GeneBLAzer® EDNRB HEK 293T DA and GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response to ET-1 under optimized conditions



GeneBLAzer® EDNRB HEK 293T DA cells and GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T 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 ET-1 in the presence of 0.5% 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 ET-1 (n=6 for each data point).

Alternate Agonist Dose Response

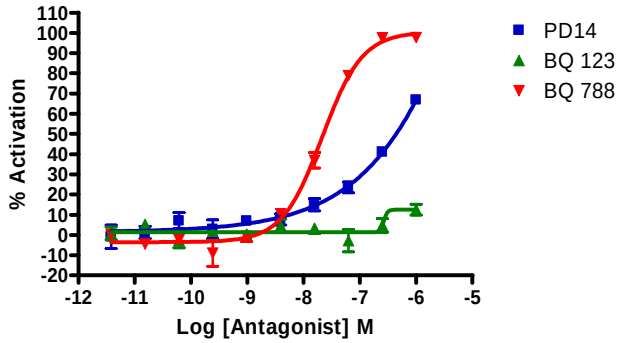
Figure 2 — GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response to ET-1, ET-2 & ET-3 under optimized conditions



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated the day before the assay in a 384-well format. On the day of the assay, cells were stimulated with ET-1(Calbiochem 05-23-3800), ET-2(Sigma #E9012) and ET-3 (Sigma #E9137) as a 3-fold dilution series from a maximum stimulation concentration of 0.1µM to a minimum concentration of 5pM in the presence of 0.5% 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 the % Activation shown plotted against the indicated concentrations of ET-1, ET-2 and ET-3(n=16 for each data point).

Antagonist Dose Response

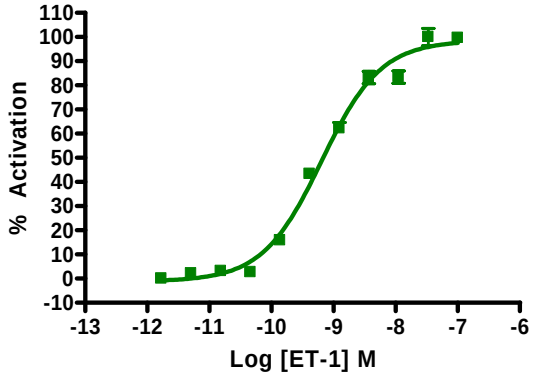
Figure 3 — GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response to PD1429893, BQ 123 and BQ788



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells were plated the day before the assay at 5,000 cells per well in a 384-well format. The antagonists PD142893 (Sigma #P2959), BQ-788 (Sigma #B-157) and BQ-123 (Sigma #B-150) were added to separate wells as a 4-fold dilution series from a maximum concentration of 1µM to a minimum concentration of 3.8pM and the cells were incubated at 37°C & 5% CO_2 for 45 min. ET-1 was then added to the plate at the EC_{50} concentration of 125 pM along with 0.5% DMSO**.** Cells were incubated for 5 hours and 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 % Inhibition is shown plotted against the indicated concentrations of the antagonists. BQ-123 is an EDNRA selective antagonist, BQ-788 is an EDNRB selective antagonist, and PD142893 is a non-selective antagonist.

Agonist Dose Response using Fluo-4NW

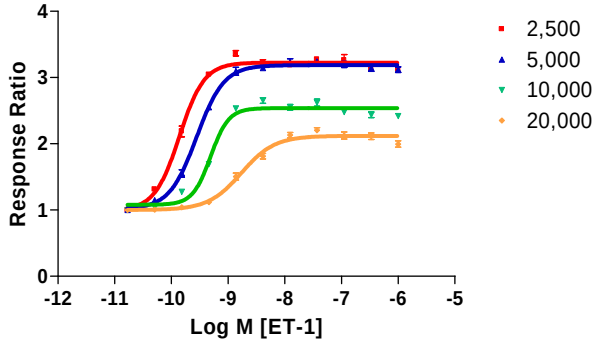
Figure 4 — GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response ET-1 using Fluo-4NW



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated the day before the assay in a 384-well format. Cells were then incubated with Fluo-4NW for 30 min. at 37°C, followed by 30 min. at room temperature. Cells were then stimulated with a dilution series of ET-1 (Calbiochem 05-23-3800) in the presence of 0.5% DMSO. Fluorescence emission values at 516 nm were obtained and the % Activation plotted against the indicated concentrations of agonist (n=16 for each data point).

Assay Performance with Variable Cell Number

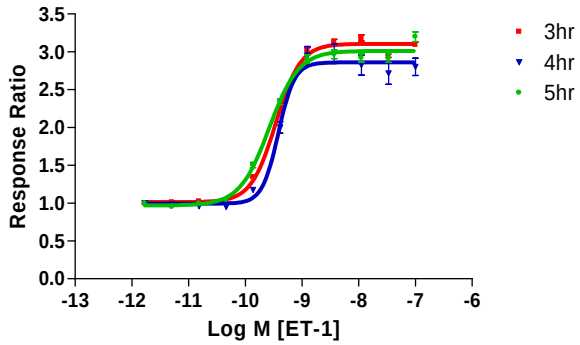
Figure 5— GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response using 2.5K, 5K, 10K and 20K cells



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells were plated the day before the assay at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format in DMEM+10% FBS. On the day of the assay, cells were stimulated with ET-1(Calbiochem #05-23-3800) in the presence of 0.5% 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 cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of ET-1 (n=8 for each data point).

Assay Performance with Variable Stimulation Time

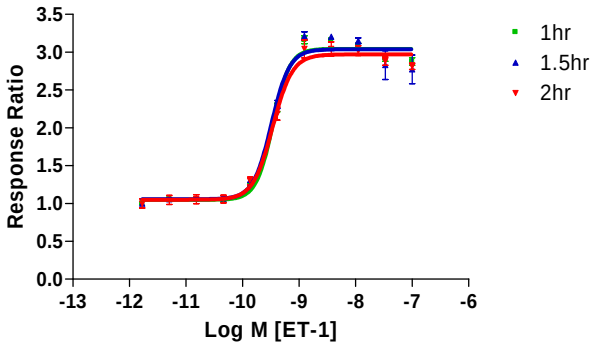
Figure 6 – GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response using 3, 4, or 5 hr stimulation times



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated the day before the assay in a 384-well format in DMEM+10% FBS. ET-1(Calbiochem #05-23-3800) was then added to the plate as a 3- fold dilution series from a maximum stimulation concentration of 0.1µM to a minimum concentration of 5pM. Plates were stimulated for 3, 4, or 5 hrs with ET-1 in 0.5% DMSO and 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 for the indicated concentrations of ET-1 (n=16 for each data point).

Assay Performance with Variable Substrate Loading Times.

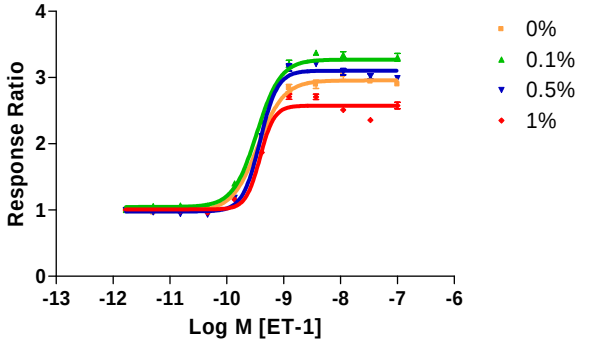
Figure 7 – GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response using 1, 1.5, and 2hr substrate loading times



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells were plated the day before the assay at 5,000 cells/well in a 384-well format in DMEM+10% FBS. On the day of the assay, cells were stimulated with ET-1(Calbiochem #05-23-3800) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 1, 1.5 or 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of ET-1 (n=16 for each data point).

Assay Performance with Variable DMSO Concentrations

Figure 8 – GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T dose response using 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® EDNRB-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate in DMEM+10% FBS. ET-1(Calbiochem 05-23-3800) was then added to the plate as a 3- fold dilution series from a maximum stimulation concentration of 0.1µM to a minimum concentration of 5pM. DMSO was added to the assay at concentrations from 0% to 1%. Plates were stimulated for 5 hrs and 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 for each DMSO concentration against the indicated concentrations of ET-1 (n=8 for each data point).

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