

Validation & Assay Performance Summary



GeneBLAzer[®] ER-beta DA Cells & Assay Kit

GeneBLAzer[®] ER-beta UAS-*bla* GripTite[™] 293 Cells

Cat. no. K1399, K1689

Target Description

Estrogen receptor beta (ER beta) is an important member of the nuclear hormone receptor superfamily. It plays a critical role in breast cancer as well as in other women's health issues.

Cell Line Description

GeneBLAzer[®] ER beta DA (Division Arrested) cells and ER beta-UAS-*bla* GripTite[™] 293 cells contain the ligand-binding domain (LBD) of the human Estrogen receptor beta (ER beta) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer[®] UAS-*bla* GripTite[™] 293 cell line. GeneBLAzer[®] UAS-*bla* GripTite[™] 293 cells stably express a beta-lactamase reporter gene under the transcriptional control of an upstream activator sequence (UAS). When an agonist binds to the LBD of the GAL4 (DBD)-ER beta (LBD) fusion protein, the protein binds to the UAS, resulting in expression of beta-lactamase. Division Arrested (DA) cells are available in two configurations- an Assay Kit (which includes cells and sufficient substrate to analyze 1 x 384-well plate), and a tube of cells sufficient to analyze 10 x 384-well plates.

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 ER beta DA cells and ER beta-UAS-*bla* GripTite[™] 293 cells are functionally validated for Z' and EC₅₀ concentrations of 17-beta Estradiol (Figure 1). In addition, ER beta-UAS-*bla* GripTite[™] 293 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 available.

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLazer™-FRET B/G Substrate.

1. Primary agonist dose response under optimized conditions (n=6)

	<u>DA</u>	<u>Dividing</u>
17-β-Estradiol (E2) EC ₅₀	0.6nM	0.5nM
Z'-Factor (EC ₁₀₀)	0.77	0.88

Response Ratio = 11 (dividing), 25 (DA)
Optimum cell no. = 20K cells/well
Optimum [DMSO] = up to 0.5%
Stimulation Time = 16 hours
Max. [Stimulation] = 30 nM

2. Alternate agonist dose response

See agonist dose response section

3. Antagonist dose response

See antagonist dose response section

4. Cell culture and maintenance

See Cell Culture and Maintenance Section and Table 1

Assay Testing Summary

5. Assay performance with variable cell number

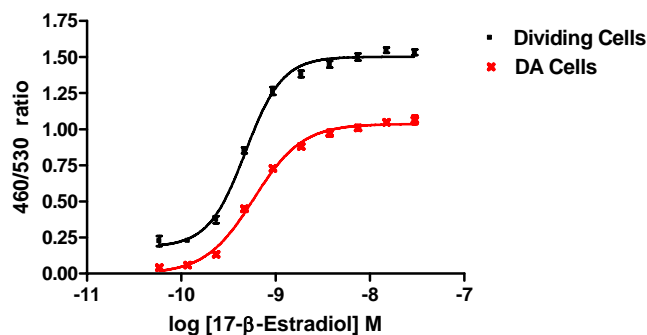
6. Assay performance with variable DMSO concentration

7. Assay performance with variable stimulation time

8. Assay performance with variable substrate loading time

Primary Agonist Dose Response

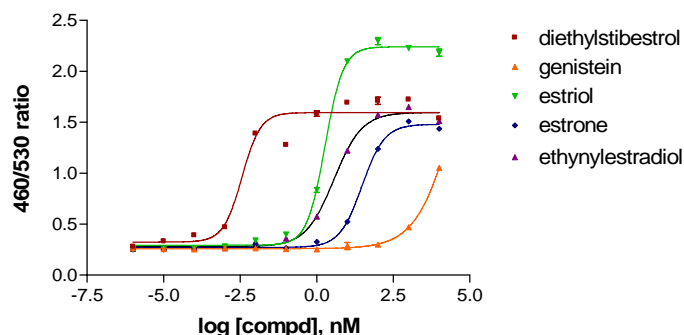
Figure 1 — ER beta DA and ER beta-UAS-*bla* GripTite™ 293 dose response to 17-beta Estradiol under optimized conditions



ER beta DA cells and ER beta-UAS-*bla* GripTite™ 293 cells (20,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of 17-beta Estradiol in the presence of 0.5% DMSO for 16 hours. Cells were then loaded with LiveBLazer™-FRET B/G Substrate (1μM final concentration of CCF4-AM) for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios plotted for each replicate against the concentrations of 17-beta Estradiol (n=6 for each data point).

Alternate Agonist Dose Response

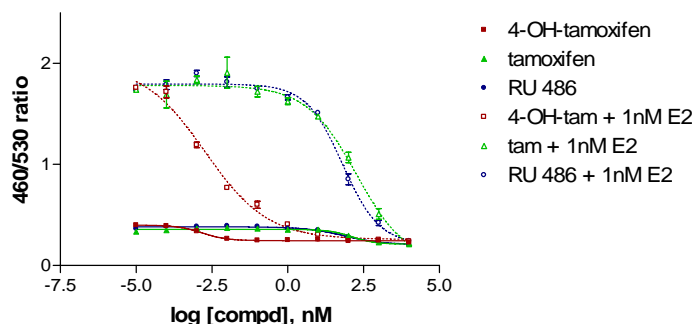
Figure 2 — ER beta-UAS-*bla* GripTite™ 293 dose response to alternate agonists



ER beta-UAS-*bla* GripTite™ 293 cells were starved overnight and plated the day of the assay in a 384-well black-walled tissue culture assay plate (20,000 cells/well). Cells were stimulated with either diethylstilbestrol, genistein, estriol, estrone, or ethynylestradiol over the indicated concentration range in the presence of 0.5% DMSO for 16 hours. Cells were then loaded with LiveBLazer™-FRET B/G Substrate (1μM final concentration of CCF4-AM) for 90 minutes. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of the agonists. (n=4 for each data point).

Antagonist Dose Response

Figure 3 — ER beta-UAS-*bla* GripTite™ 293 dose response to known SERMS RU486, Tamoxifen, and 4-Hydroxytamoxifen



Selective Estrogen Receptor Modulators (SERMS) were tested in both agonist and antagonist mode. ER beta-UAS-*bla* GripTite cells were starved overnight and then plated (20,000 cells/well) in a 384-well black-walled tissue culture assay plate. For antagonist mode, cells were treated with RU486, Tamoxifen, and 4-Hydroxytamoxifen and incubated at 37 degrees C for 30 min. followed by 1 nM E2 agonist stimulation for 16 hours in 0.5% DMSO. For agonist mode, the cells were treated similarly, except no E2 was added. Cells were then loaded for 90 minutes 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 Ratios are shown plotted against the indicated concentrations of ligand (n=4 for each data point).

Dividing Cell Culture and Maintenance

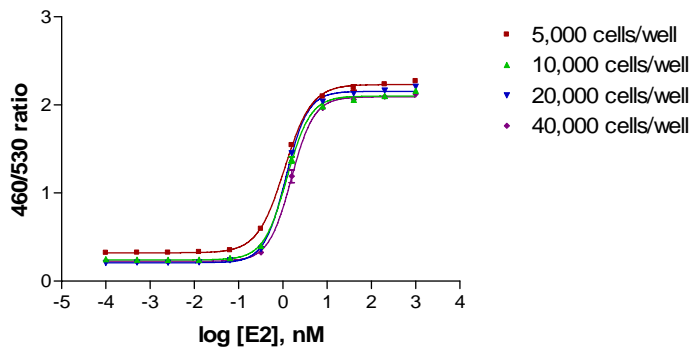
Dividing cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37°C and 5% CO₂. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

Table 1 – Dividing Cell Culture and Maintenance

Component	Growth Medium (-)	Growth Medium (+)	Assay Medium	Freeze Medium
DMEM, w/ GlutaMAX™	90%	90%	—	—
Phenol Red free DMEM	—	—	98%	—
Dialyzed FBS Do not substitute!	10%	10%	—	—
Charcoal/Dextran FBS			2%	
NEAA	0.1 mM	0.1 mM	0.1 mM	—
Sodium Pyruvate	1 mM	1 mM	1 mM	—
Hygromycin	—	80 µg/mL	—	—
Zeocin®	—	80 µg/mL	—	—
Penicillin	100 U/mL	100 U/mL	100 U/mL	—
Streptomycin	100 µg/mL	100 µg/mL	100 µg/mL	—
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

Assay Performance with Variable Cell Number

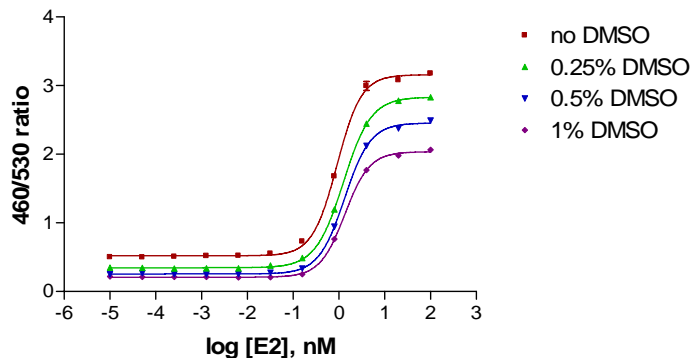
Figure 4 – ER beta-UAS-*bla* GripTite dose response to E2 with 5, 10, 20 and 40K cells per well



ER beta-UAS-*bla* GripTite cells were starved overnight and then plated at 5,000, 10,000, 20,000 or 40,000 cells/well in a 384-well black-walled tissue culture assay plate. Cells were stimulated with E2 for 16 hours in the presence of 0.5% DMSO. Cells were then loaded with LiveBLazer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for 90 minutes. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of E2 (n=8 for each data point).

Assay Performance with variable DMSO concentration

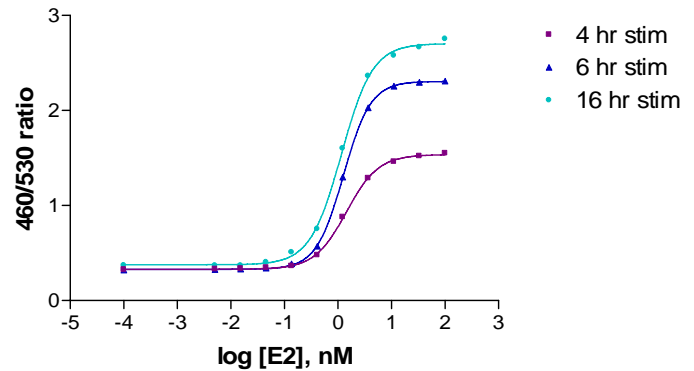
Figure 5 – ER beta-UAS-*bla* GripTite dose response to E2 with 0, 0.25, 0.5 and 1% DMSO.



ER beta-UAS-*bla* GripTite cells were starved overnight and then plated at 20,000 cells/well in a 384-well black-walled tissue culture assay plate. E2 was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 16 hrs with agonist and loaded for 2 hours with LiveBLazer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios are shown plotted for each DMSO concentration against the indicated concentrations of E2 (n=8 for each data point).

Assay Performance with variable stimulation time

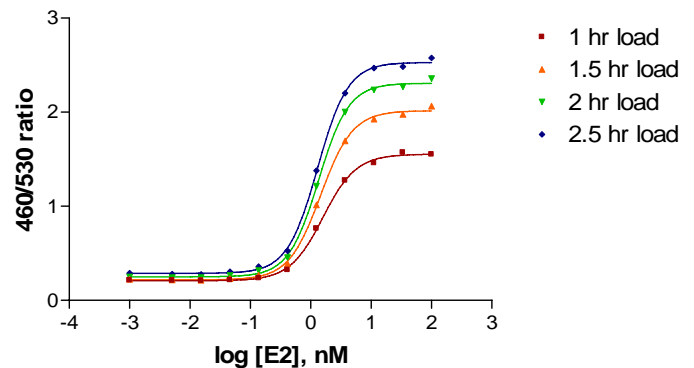
Figure 6 – ER beta-UAS-*bla* GripTite dose response to E2 with 4, 6, and 16 hour stimulation times



ER beta-UAS-*bla* GripTite cells were starved overnight and then plated at 20,000 cells/well in a 384-well black-walled tissue culture assay plate. Cells were stimulated with E2 in the presence of 0.5% DMSO for 4, 6, and 16 hours and then loaded for 90 minutes with LiveBLazer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of E2 (n=16 for each data point).

Assay Performance with variable substrate loading time

Figure 7 – ER beta-UAS-*bla* GripTite dose response to E2 with 60, 90, 120 and 150 minute substrate loading time



ER beta-UAS-*bla* GripTite cells were starved overnight and then plated at 20,000 cells/well in a 384-well black-walled tissue culture assay plate. Cells were stimulated with E2 for 16 hours in the presence of 0.5% DMSO. Cells were then loaded with LiveBLazer™-FRET B/G Substrate (1 μ M final concentration of CCF4-AM) for either 60, 90, 120, or 150 minutes. Fluorescence emission values at 460 nm and 530 nm for the various loading times were obtained using a standard fluorescence plate reader and the Ratios plotted against the indicated concentrations of E2 (n=16 for each data point).