Validation & Assay Performance Summary

GeneBLAzer® AR-UAS-*bla* GripTite™ Cells

Cat. no. K1698

Target Description

The androgen receptor (AR) is a validated drug target of the nuclear receptor superfamily. AR antagonists like flutamide and bicalutamide are used in the treatment of androgen-dependent prostate cancer.

Cell Line Description

GeneBLAzer® AR-UAS-*bla* GripTite™ 293 cells contain the ligand-binding domain (LBD) of the rat Androgen receptor (AR) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-*bla* GripTite™ 293 cell line. This portion of the rat AR is identical to the human AR in the conserved LBD and differs from the human sequence at five residues in the hinge region. GeneBLAzer® UAS-*bla* GripTite™ 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)-AR (LBD) fusion protein, the protein binds to the UAS, resulting in expression of beta-lactamase.

GeneBLAzer® AR-UAS-*bla* GripTite™ 293 cells are functionally validated for Z' and EC_{50} concentrations of R1881 (Figure 1). In addition, AR-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 a 384-well format using LiveBLAzer™-FRET B/G Substrate.

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

R1881 EC_{50}	= 0.4 nM
Z'-Factor (EC_{100})	= 0.68
Response Ratio	= 3.4
Optimum cell no.	= 8K 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**

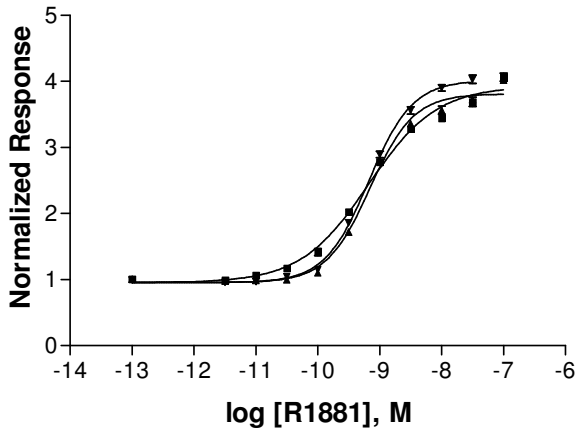
6. **Assay performance with variable DMSO concentration**

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

8. **Assay performance with variable substrate loading time**

Primary Agonist Dose Response

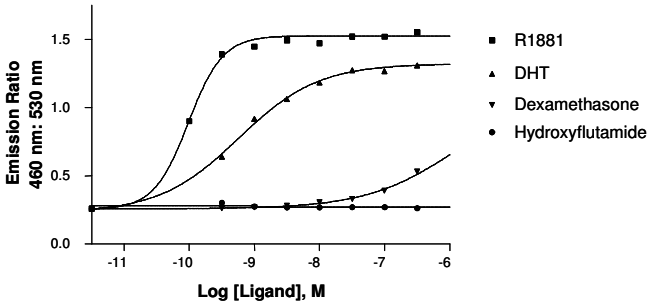
Figure 1 — AR-UAS-*bla* GripTite™ dose response to known agonist R1881



AR-UAS-*bla* GripTite™ 293 cells (8,000 cells/well) were plated in a 384-well format and serum starved for 24 hours. Cells were then stimulated with a dilution series of R1881 in the presence of 0.5% DMSO for 16 hours. Cells were subsequently loaded with LiveBLAzer™-FRET B/G Substrate (1 µM 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 date from three individual users were plotted for each replicate against the concentrations of R1881.

Alternate Agonist Dose Response

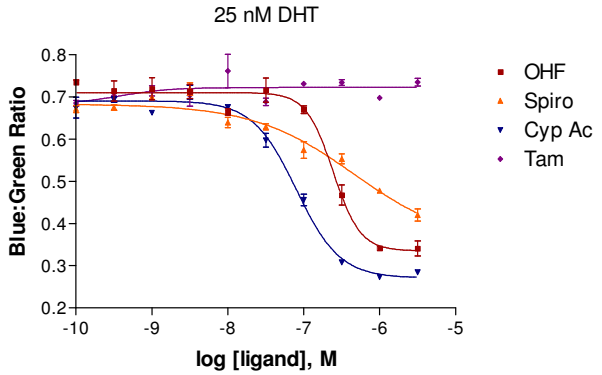
Figure 2 — AR-UAS-*bla* GripTite™ dose response to known agonists R1881 and DHT



AR-UAS-*bla* GripTite™ cells (8,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either R1881, DHT (dihydrotestosterone), dexamethasone, or hydroxyflutamide 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 (1 µM 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 were plotted against the indicated concentrations of the agonists.

Antagonist Dose Response

Figure 2 — AR-UAS-*bla* GripTite™ dose response to known antagonists OHF, Spironolactone and Cyproterone Acetate



AR-UAS-*bla* GripTite™ cells (8,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with OHF (hydroxyflutamide), Spironolactone, Cyproterone Acetate and tamoxifen (as a specificity control) and incubated at 37 degrees C for 45 min., followed by 25 nM DHT (dihydrotestosterone) agonist stimulation for 16 hours in 0.5% DMSO. Cells were then loaded for 120 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 were plotted against the indicated concentrations of ligand.

Cell Culture and Maintenance

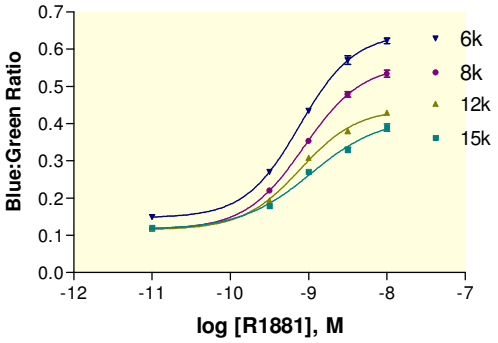
Cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37ºC and 5% CO_2. Split cells at least twice a week. Do not allow cells to reach confluence.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	Freeze Medium
DMEM, w/ GlutaMAX™	90%	90%	—	—
Opti-MEM	—	—	90%	—
Dialyzed FBS **Do not substitute!**	10%	10%	10%	—
NEAA	0.1 mM	0.1 mM	0.1 mM	—
Sodium Pyruvate	—	—	1 mM	—
HEPES (pH 7.3)	25 mM	25 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

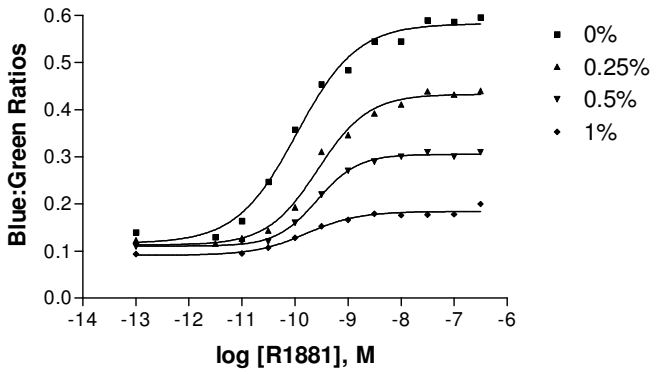
Figure 3 – R1881 dose response with 6, 8, 12 and 15K cells per well



AR-UAS-*bla* GripTite™ cells were plated at 6,000, 8,000, 12,000 or 15,000 cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with R1881 for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) 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 ratios were plotted against the indicated concentrations of R1881.

Assay Performance with variable DMSO concentration

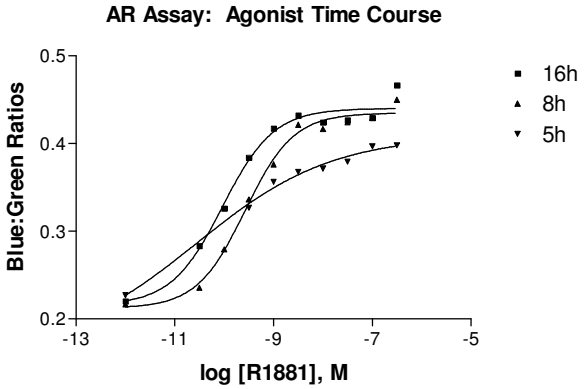
Figure 4 – AR-UAS-*bla* GripTite™ dose response to R1881 with 0, 0.25, 0.5 and 1% DMSO.



AR-UAS-*bla* GripTite™ cells (8,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. R1881 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 µM 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 were plotted for each DMSO concentration against the indicated concentrations of R1881.

Assay Performance with variable stimulation time

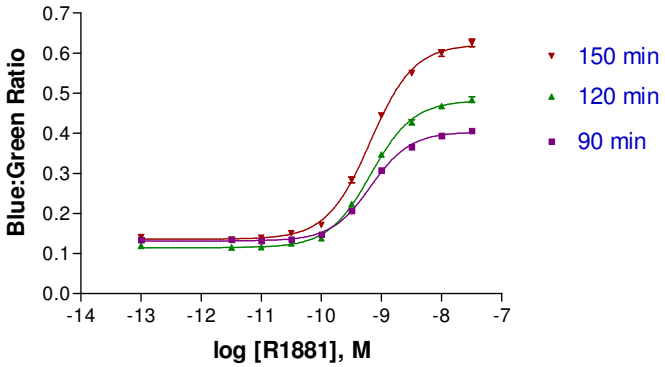
Figure 5 – AR-UAS-*bla* GripTite™ dose response to R1881 with 5, 8, and 16 hour stimulation times



AR-UAS-*bla* GripTite™ cells (8,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. R1881 was then added to the plate over the indicated concentration range for 5, 8, and 16 hours and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (1 µM 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 were plotted against the indicated concentrations of R1881.

Assay Performance with variable substrate loading time

Figure 6 – AR-UAS-*bla* GripTite™ dose response to R1881 with 90, 120 and 150 minute substrate loading time



AR-UAS-*bla* GripTite™ cells were plated at 8,000 cells/well in a 384-well format the day of the assay in 0.5% DMSO. Cells were stimulated with R1881 for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 µM final concentration of CCF4-AM) for either 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 were plotted against the indicated concentrations of R1881.