Validation & Assay Performance Summary

GeneBLAzer® PR DA Assay Kit

GeneBLAzer® PR DA Cells

GeneBLAzer® PR-UAS-*bla* HEK293T Cells

Cat. no. K1403, K1693

Cell Line Descriptions

GeneBLAzer® PR DA (Division Arrested) cells and PR-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Progesterone Receptor (PR) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer® UAS-*bla* HEK 293T cell line. GeneBLAzer® UAS-*bla* HEK 293T 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)-PR (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 PR DA cells and PR-UAS-*bla* HEK 293T cells are functionally validated for Z' and EC_{50} concentrations of R5020. In addition, PR-UAS-*bla* HEK 293T cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time (data available upon request). Additional testing data using alternate stimuli are also available.

Target Description

The progesterone receptor (PR) is a member of the family of ligand-inducible nuclear receptors that regulate hormone-responsive genes by altering the rate of transcription initiation. Progesterone has been implicated in a variety of hormone-dependant cancers.

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=3)**

	DA Cells	Dividing Cells
R5020 EC_{50}	293 pM	313 pM
Z'-Factor	0.92	0.89

Response Ratio = 7.3 – 11.0
Optimum cell no. = 15K cells/well
Optimum [DMSO] = up to 0.5%
Stimulation Time = 16 hours
Max. [Stimulation] = 100 nM

2. **Alternate agonist dose response**

Progesterone EC_{50} = 3.7 – 10.0 pM
Z'-Factor (EC_{100}) = 0.78 – 0.90
Response Ratio = 6.9 – 8.4
Max. [Stimulation] = 300 nM

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

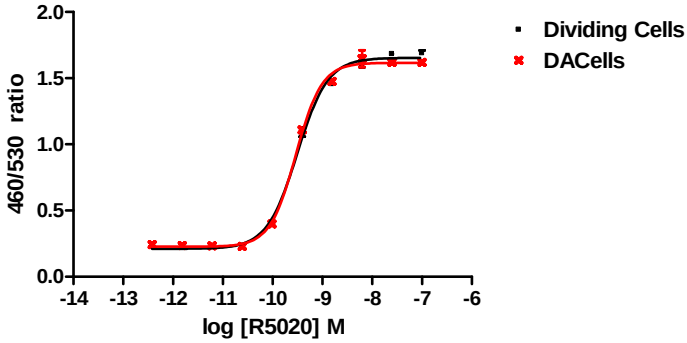
5. **Assay performance with variable cell number**

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

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

Primary Agonist Dose Response

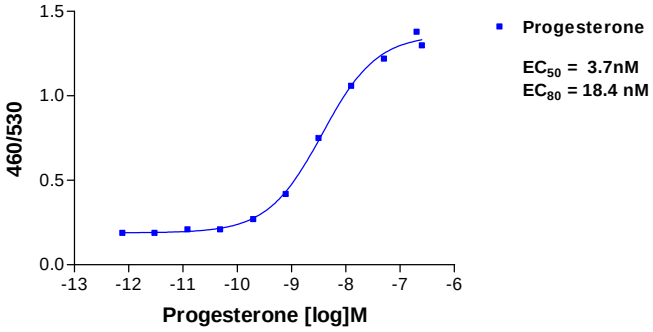
Figure 1 — PR DA and PR-UAS-*bla* HEK 293T dose response to R5020 under optimized conditions



PR DA cells and PR-UAS-*bla* HEK 293T cells (15,000 cells/well) were plated in a 384-well format and stimulated with a dilution series of R5020 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 plotted for each replicate against the concentrations of R5020 (n=6 for each data point).

Alternate Agonist Dose Response

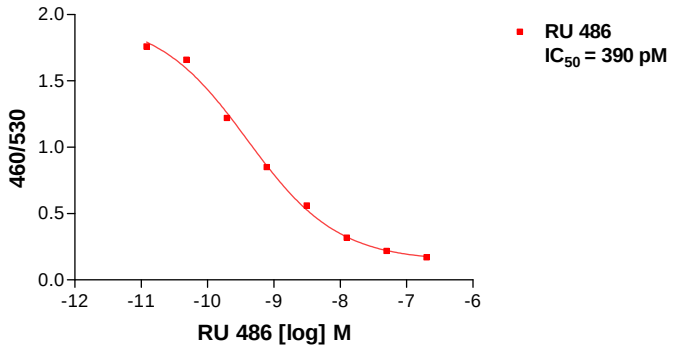
Figure 2 — PR-UAS-*bla* HEK 293T dose response to known agonist progesterone



PR-UAS-*bla* HEK 293T cells (15,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with progesterone 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 120 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 progesterone.

Antagonist Dose Response

Figure 2 — PR-UAS-*bla* HEK 293T dose response to known antagonist RU486



PR-UAS-*bla* HEK 293T cells (15,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with RU486 and incubated at 37 degrees C for 45 min., followed by 0.5 nM R5020 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 are shown plotted against the indicated concentrations of ligand.

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 37ºC and 5% CO_2. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

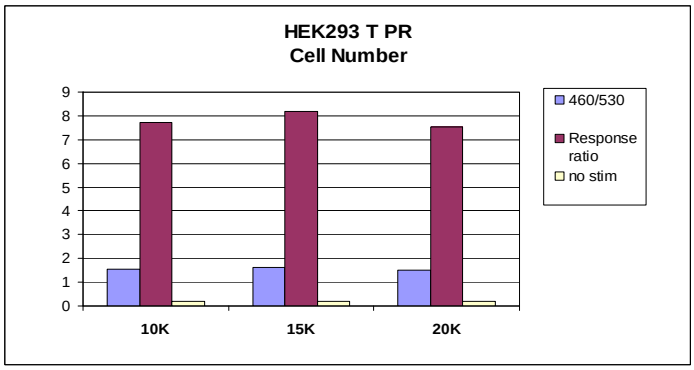
Cells are adherent and require 1X Matrigel.

Table 1 – Cell Culture and Maintenance (Dividing Cells)

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	1X Matrigel	Freeze Medium
DMEM	90%	90%	—	99.75%	—
DMEM w/o Phenol Red	—	—	98%	—	—
Dialyzed FBS **Do not substitute!**	10%	10%	—	—	—
Charcoal Stripped FBS	—	—	2%	—	—
NEAA	0.1 mM	0.1 mM	—	—	—
Sodium Pyruvate	1 mM	1 mM	—	—	—
HEPES (pH 7.3)	25 mM	25 mM	—	—	—
Hygromycin	—	100 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	—	—
Matrigel	—	—	—	0.25%	—
Recovery™ Cell Culture Freezing Medium	—	—	—	—	100%

Assay Performance with Variable Cell Number

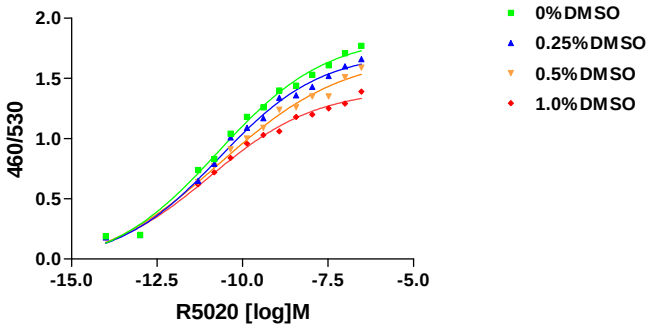
Figure 3 – Response with 10, 16, and 20K cells per well



PR-UAS-*bla* HEK 293T cells were plated at 10,000, 16,000, or 20,000 cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with R5020 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of R5020.

Assay Performance with variable DMSO concentration

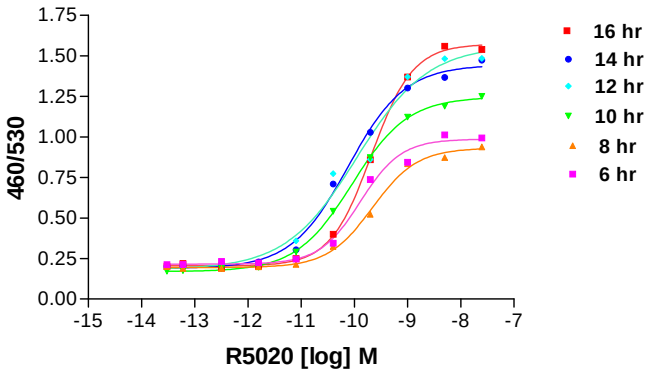
Figure 4 – PR-UAS-*bla* HEK 293T dose response to R5020 with 0, 0.25, 0.5 and 1% DMSO.



PR-UAS-*bla* HEK 293T cells (15,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. R5020 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 (1µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios are shown plotted for each DMSO concentration against the indicated concentrations of R5020.

Assay Performance with variable stimulation time

Figure 5 – PR-UAS-*bla* HEK 293T dose response to R1881 with 6, 8, 10, 12, 14 and 16 hour stimulation times



PR-UAS-*bla* HEK 293T cells (15,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. R5020 was then added to the plate over the indicated concentration range for 6, 8, 10, 12, 14, or 16 hours and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of R5020.