Validation & Assay Performance Summary

GeneBLAzer® GR DA Assay Kit

GeneBLAzer® GR DA Cells

GeneBLAzer® GR-UAS-*bla* HEK 293T Cells

Cat. no. K1391, K1687

Target Description

The glucocorticoid receptor (GR) is a validated drug target for inflammation. GR targets such as dexamethasone are clinically available as anti-inflammatory drugs.

Cell Line Description

GeneBLAzer® GR DA (Division Arrested) cells and GR-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Gluocorticoid receptor (GR) 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)-GR (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 GR DA cells and GR-UAS-*bla* HEK 293T cells are functionally validated for Z' and EC_{50} concentrations of Dexamethasone (Figure 1). In addition, GR-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.

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)**

	DA	Dividing
Dexamethasone EC_{50}	1.8nM	1.7nM
Z'-Factor (EC_{100})	0.96	0.94

Response Ratio	= 15
Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 1%
Stimulation Time	= 16 hours
Max. [Stimulation]	= 100 nM

2. **Alternate agonist dose response**

Betamethasone EC_{50}	= 3.1 nM
Budesonide EC_{50}	= 0.07 nM
Cortisol EC_{50}	= 44 nM

3. **Antagonist dose response**

Mifepristone IC_{50}	= 0.16 nM
Progesterone IC_{50}	= 39 nM
Cortisone IC_{50}	= 820 nM

4. **Cell culture and maintenance**
   See Cell Culture and Maintenance Section and Table 1

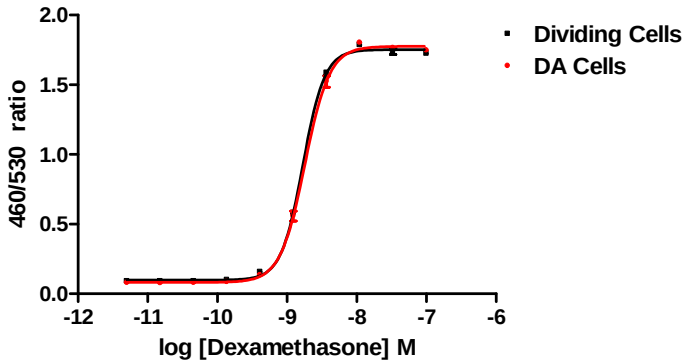
Assay Testing Summary

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

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

Primary Agonist Dose Response

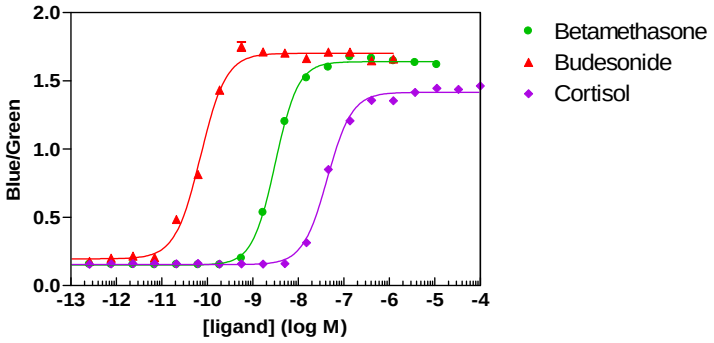
Figure 1 — GR DA and GR-UAS-*bla* HEK 293T dose response to Dexamethasone under optimized conditions



GR DA cells and GR-UAS-*bla* HEK 293T cells (20,000 cells/well) were plated in a 384-well format stimulated with a dilution series of Dexamethasone 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 Dexamethasone (n=6 for each data point).

Alternate Agonist Dose Response

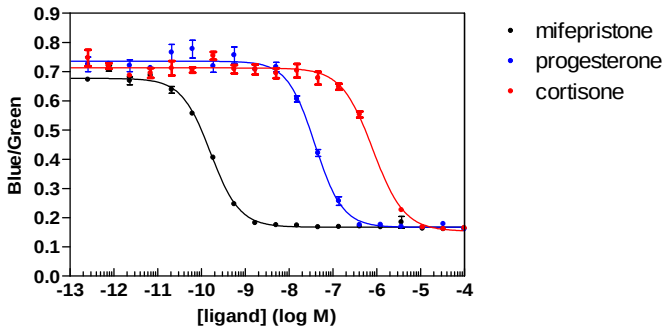
Figure 2 — GR-UAS-*bla* HEK 293T dose response to known agonists betamethasone, budesonide and cortisol



GR-UAS-*bla* HEK 293T Cells were treated as per the customer protocol. Dose response curves were performed with alternate agonists as shown above. EC_{50} values are budesonide 0.07 nM, betamethasone 3.1 nM, and cortisol 44 nM.

Antagonist Dose Response

Figure 3 — GR-UAS-*bla* HEK 293T dose response to known antagonists mifepristone, progesterone and cortisone



GR-UAS-*bla* HEK 239T Cells were treated as per the customer protocol. Dose response curves were carried out with several antagonists. The antagonists were incubated in the presence of EC_{80} amounts of dexamethasone. IC_{50} values are Mifepristone (RU486) 0.16 nM, progesterone 39 nM, cortisone 820 nM.

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.

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	—
HEPES (pH 7.3)	25 mM	25 mM	—	—
Hygromycin B	—	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	—
Sodium Pyruvate	—	—	1 mM	
Recovery™ Cell Culture Freezing Medium	—	—	—	100%

Assay Performance with Variable Cell Number

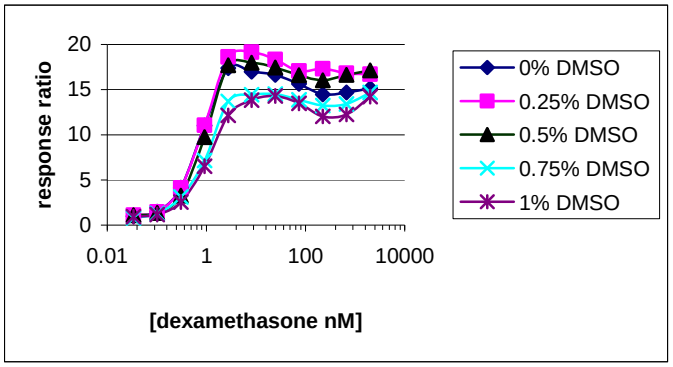
Table 2 – Effect of variations in cell number

Cells/well	Response Ratio	Z'
2000	11.2	0.79
5000	13.9	0.86
10,000	16.3	0.91
20,000	17.9	0.94
40,000	18.4	0.9

GR-UAS-*bla* HEK 293T Cells were treated as per the customer protocol, except the number of cells per well was varied. The effect of variations in cell number on Z'-factor values and Response Ratio was tested in 384-well plate format.

Assay Performance with variable DMSO concentration

Figure 4 – GR-UAS-*bla* HEK 293T dose response to dexamethasone with 0, 0.25, 0.5, 0.75 and 1% DMSO.



GR-UAS-*bla* HEK 293T Cells were treated as per the customer protocol in the presence of various concentrations of DMSO. A typical dose reponse experiment to dexamethasone was carried out in a 96 well plate.