Validation & Assay Performance Summary

GeneBLAzer® LXR-beta DA Cells and Assay Kit

GeneBLAzer® LXR beta-UAS-*bla* HEK 293T Cells

Cat. no. K1415 and K1699

Target Description

LXR beta (liver X receptor beta) is a nuclear hormone receptor that regulates transcription involved in the metabolism of cholesterol and fatty acids. Activation of LXR beta induces reverse cholesterol transport and increases HDL cholesterol *in vivo*. It is a validated drug target for cardiovascular disease, and has been implicated in diabetes, inflammation and neurodegenerative disease. LXR beta and LXR alpha have a high degree of homology, but have very different tissue distribution. While LXR alpha is expressed in metabolically active tissue (such as the liver, kidney, intestine, adipose tissue, and macrophages), LXR beta is ubiquitously expressed. Both LXR beta and LXR alpha function by forming heterodimers with RXR and subsequently binding to specific DNA response elements within the regulatory regions of their target genes.

Cell Line Description

GeneBLAzer® LXR beta DA (Division Arrested) cells and LXR beta-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Liver-X receptor-beta (LXR beta) 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)-LXR 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 LXR beta DA cells and LXR beta-UAS-*bla* HEK 293T 293 cells are functionally validated for Z' and EC_{50} concentrations of TO901317 (Figure 1). In addition, LXR beta-UAS-*bla* HEK 293T 293 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
TO901317 EC_{50}	47 nM	58nM
Z'-Factor (EC_{100})	0.67	0.60

Response Ratio = 4.4
Optimum cell no. = 10K cells/well
Optimum [DMSO] = up to 1%
Stimulation Time = 16 hours

2. **Alternate agonist dose response**
   See agonist dose response section

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

Assay Testing Summary

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

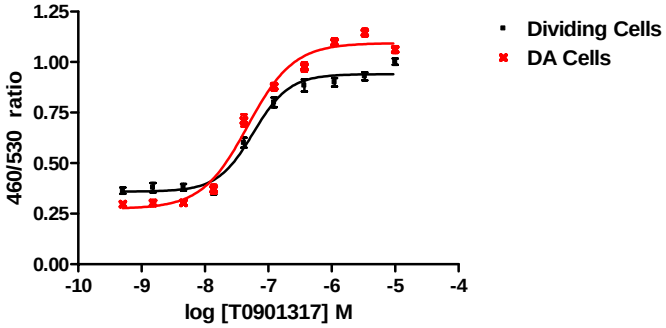
5. **Assay performance with variable DMSO concentration**

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

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

Primary Agonist Dose Response

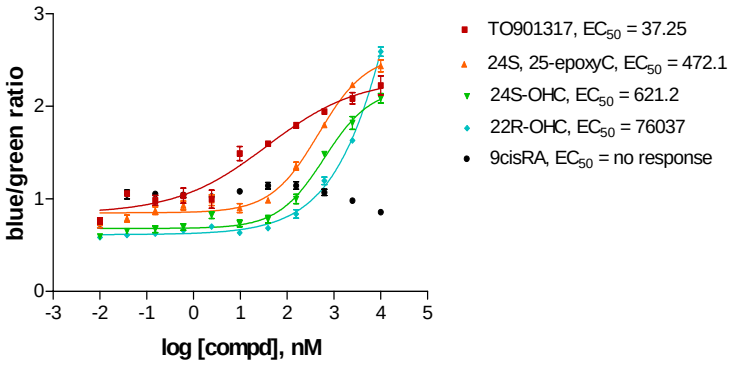
Figure 1 — LXR beta-UAS-*bla* HEK 293T dose response to known agonist TO901317



LXR beta DA cells and LXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated in a 384-well format and serum starved for 24 hours. Cells were then stimulated with a dilution series of TO901317 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 TO901317 (n=6 for each data point).

Alternate Agonist Dose Response

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



LXR beta-UAS-*bla* HEK 293T cells were starved for 24hrs, then (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either TO901317, 24S, 25-epoxyC, 24S-OHC, 22R-OHC or 9-*cis* retinoic acid 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 the agonists (n= 8 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 37ºC and 5% CO_2. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

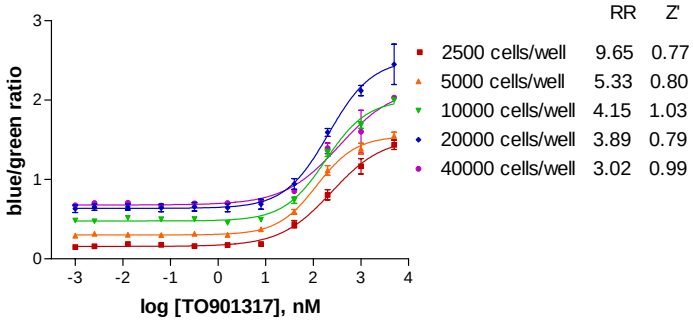
All flasks must be pre-coated with 1X matrigel prior to plating cells.

Table 1 – Cell Culture and Maintenance

Component	Growth Medium (–)	Growth Medium (+)	Assay Medium	1X Matrigel
DMEM, w/ GlutaMAX™	90%	90%	—	99.75%
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	—
HEPES (pH 7.3)	25 mM	25 mM	—	—
Hygromycin	—	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%

Assay Performance with Variable Cell Number

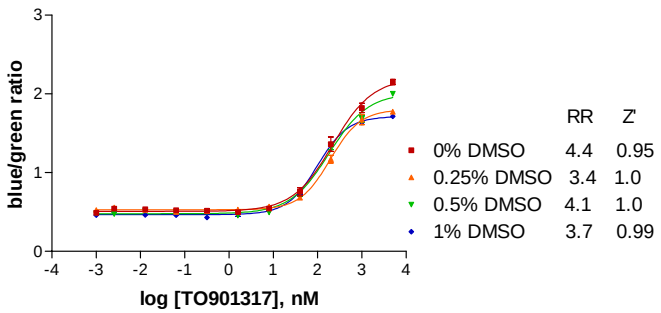
Figure 3 – TO901317 dose response with 1.5, 5, 10, 20 and 40K cells per well



LXR beta-UAS-*bla* HEK 293T cells were serum starved for 24 hrs, then plated at 2500, 5000, 10,000, 20,000 or 40,000 or cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with TO901317 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 TO901317 (n=8 for each data point).

Assay Performance with variable DMSO concentration

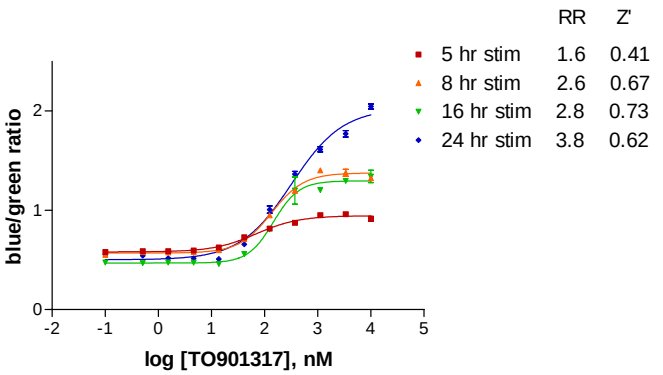
Figure 4 – LXR beta-UAS-*bla* HEK 293T dose response to TO901317 with 0, 0.25, 0.5 and 1% DMSO.



LXR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were serum starved for 24 hrs, then plated the day of the assay in a 384-well black-walled tissue culture assay plate. TO901317 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 ATRA (n=8 for each data point).

Assay Performance with variable stimulation time

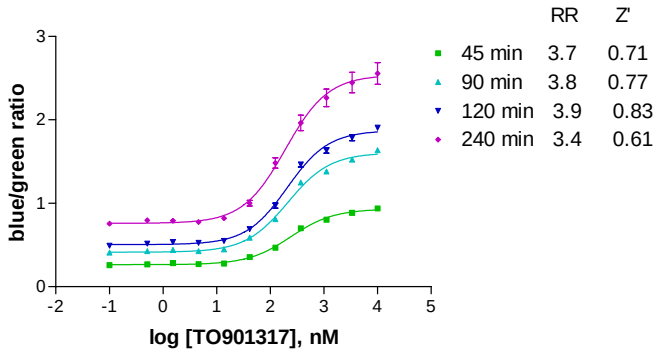
Figure 5 – LXR beta-UAS-*bla* HEK 293T dose response to TO901317 with 5, 8, 16 and 24 hour stimulation times



LXR beta-UAS-*bla* HEK 293T cells (10,000cells/well) were serum starved for 24 hrs, then plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. TO901317 was then added to the plate over the indicated concentration range for 5, 8, 16, and 24 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 ATRA (n=8 for each data point)

Assay Performance with variable substrate loading time

Figure 6 – LXR beta-UAS-*bla* HEK 293T dose response to TO901317 with 45, 90, 120 and 240 minute substrate loading time



LXR beta-UAS-*bla* HEK 293T cells were serum starved for 24 hrs, then plated at 10,000 cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with TO901317 for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1µM final concentration of CCF4-AM) for either 45, 90, 120, or 240 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 Response Ratios plotted against the indicated concentrations of TO901317(n=8 for each data point).

References

1. Zlokarnik G, Negulescu PA, Knapp TE, Mere L, Burres N, Feng L, Whitney M, Roemer K, Tsien RY. "Quantitation of transcription and clonal selection of single living cells with beta-lactamase as reporter." Science. 1998 Jan 2;279(5347):84-8.

2. Chin J, Adams AD, Bouffard A, Green A, Lacson RG, Smith T, Fischer PA, Menke JG, Sparrow CP, Mitnaul LJ. "Miniaturization of cell-based beta-lactamase-dependent FRET assays to ultra-high throughput formats to identify agonists of human liver X receptors." Assay and Drug Development Technologies. Volume 1, Number 6, 2003: 777-787.