Validation & Assay Performance Summary

GeneBLAzer® LXR alpha DA Assay Kit

GeneBLAzer® LXR alpha DA Cells

GeneBLAzer® LXR alpha -UAS-*bla* HEK293T Validated Assay

Cat. no. K1401, K1692

Target Description

The liver X receptor alpha (LXR alpha) is a nuclear hormone receptor and can function as a ligand inducible transcription factor capable of acting as a co-repressor and/or co-activator for gene expression. Nuclear receptors contain a series of conserved domains or regions. These domains/regions include a variable NH_2-domain (A/B region), a conserved DNA-binding domain (DBD or region C), a linker region (also called a hinge region) (region D), a ligand binding domain (LBD or region E), and in some receptors a variable COOH-terminal (region F) (1).

LXR alpha forms a heterodimer with RXR to activate transcription, and is a member of class II, which encompass receptors that are mainly (not always) localized in the nucleus even when not bound by ligand. (Class I nuclear receptors such as the steroid hormone receptors are sequestered in the cytoplasm and migrate to the nucleus upon ligand binding.) Unliganded LXR alpha interacts with co-repressors and represses transcription. When ligand is bound, the receptor undergoes conformational changes, leading to a decrease in the interaction with co-repressors and an increase in interactions with co-activators such as SRC-1 and GRIP-1, which then leads to transcriptional activation. The specificity of the co-activator interaction is determined by the particular ligand that is bound (1,3).

LXRs are expressed in a wide variety of tissues. The alpha isoform controls fatty acid synthesis and is expressed in metabolically active tissues such as the liver, kidney, adipose tissue, macrophages, and intestine. The beta isoform is ubiquitiously expressed, and controls high-density lipoprotein assembly through its control of ABCA1 expression. Synthetic LXR agonists which are not subtype-specific have been developed. Mice treated with these agonists show a decrease in atherosclerosis, but unfortunately also show an increase in fatty acid synthesis leading to triglyceride accumulation. It is believed that subtype-selective agonists might allow dissociation of the favorable cholesterol effects from the unfavorable triglyceride effects (2,3).

Cell Line Description

GeneBLAzer® LXR alpha DA (Division Arrested) cells and LXR alpha-UAS-*bla* HEK 293T 293 cells contain the ligand-binding domain (LBD) of the human Liver-X receptor-alpha(LXR alpha) 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 alpha (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 alpha DA cells and LXR alpha-UAS-*bla* HEK 293T 293 cells are functionally validated for Z' and EC_{50} concentrations of TO901317 (Figure 1). In addition, LXR alpha-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}	142nM	73nM
Z'-Factor (EC_{100})	0.68	0.76

Response Ratio	= 4.8
Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1%
Stimulation Time	= 16 hours
Max. [Stimulation]	= 3 μM

2. **Alternate agonist dose response**

22(R) OH-Cholesterol. EC_{50}	= 265 μM
24(S), 25- Epo-Chol. EC_{50}	= 66.6 μM
24(S) OH-Chol. EC_{50}	= 7.2 μM
GW3965 EC_{50}	= 749 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**

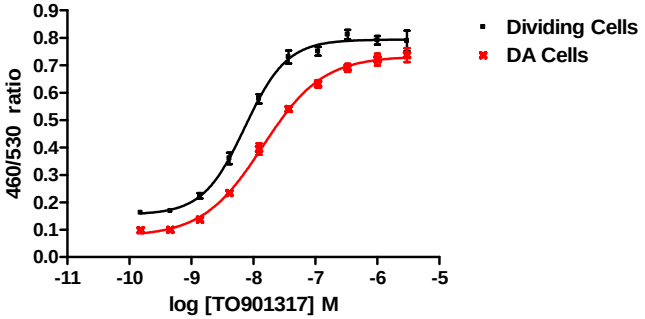
6. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

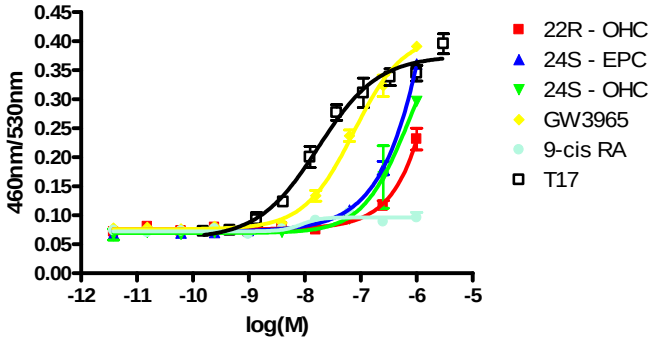
Figure 1 — LXR alpha DA and LXR alpha-UAS-*bla* HEK 293T dose response to TO901317 under optimized conditions



LXR alpha DA cells and LXR alpha-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated in a 384-well format 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

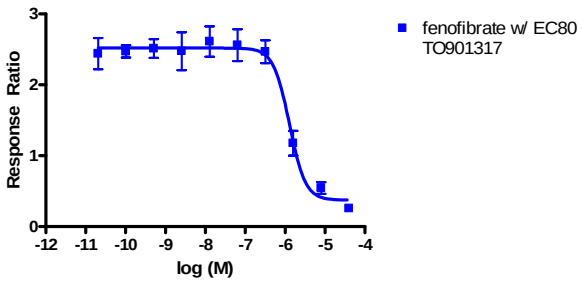
Figure 2 —Alternate agonist Dose Response



LXR alpha-UAS-*bla* HEK293T cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Cells were stimulated with either 22(R) OH-Cholesterol (Cayman # 89355), 24(S), 25- Epoxycholesterol (Biomol #GR-231), 24(S) OH-Cholesterol (Biomol #GR-230), GW3965 (Sigma #G6295), 9-cis Retinoic Acid (Biomol #GR-101), or TO901317 (Cayman #71810) 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 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Ratios plotted against the indicated concentrations of the agonists (n= 5 for each data point).

Antagonist Dose Response

Figure 3 —Fenofibrate dose response



LXR alpha-UAS-*bla* HEK293T cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with Fenofibrate (Sigma #F6020) and incubated at 37 degrees C for 30 min., followed by **75 nM** TO901317 (Cayman #71810) agonist stimulation for 16 hours. Cells were then loaded for 2 hours 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 Response Ratio is shown plotted against the indicated concentrations of the antagonist (4).

Dividing Cell Culture and Maintenance

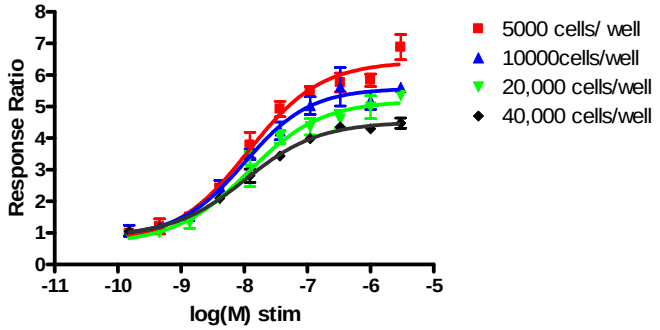
Dividing cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37ºC and 5% CO_2. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

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™	—	100 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

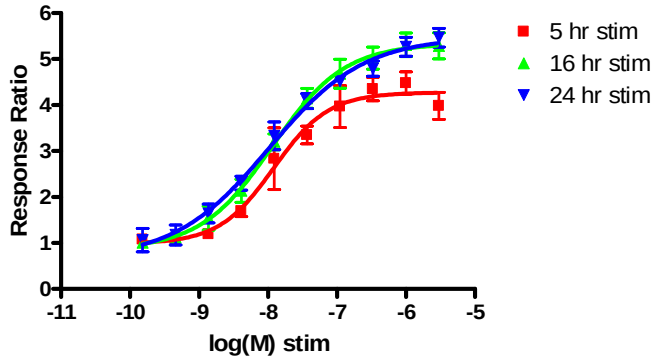
Figure 4— T0901317 dose response with 5, 10, 20, and 40K cells / well



LXR alpha-UAS-*bla* HEK293T cells were plated at, 5,000, 10,000, 20,000, or 40,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with TO901317 (Cayman chemical #71810) 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of T0901317 (n=8 for each data point).

Assay performance with Variable Stimulation Time

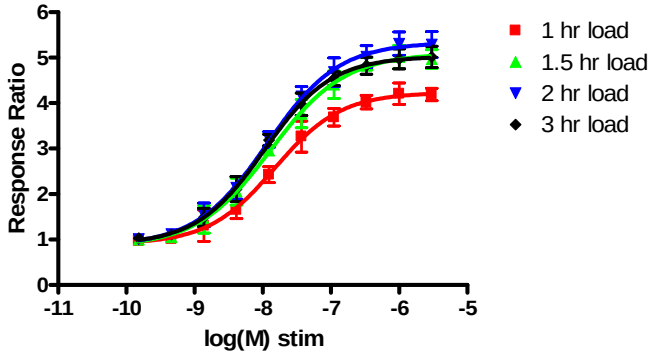
Figure 5 – T0901317 dose response with 5, 16 and 24 hour stimulation times



LXR alpha-UAS-*bla* HEK293T cells (10,000 cells/well) were plated the day of the assay in 3 separate 384-well black-walled tissue culture assay plate. TO901317 (Cayman chemical #71810) was then added to the appropriate plate over the indicated concentration range for 5, 16, and 24 hrs in 0.5% DMSO 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 (n=8 for each data point).

Assay performance with Variable Substrate Loading Time

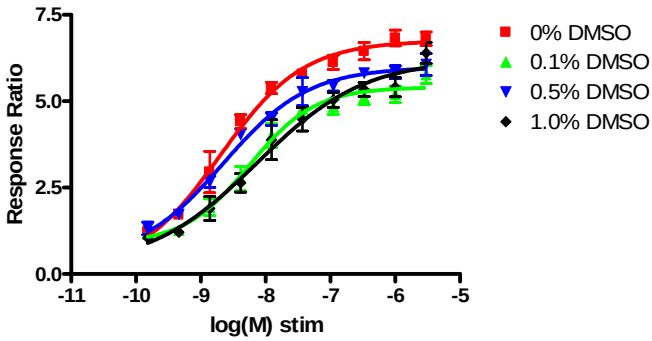
Figure 6 – T0901317 dose response with 1, 1.5, 2 and 3 hour loading times



LXR alpha-UAS-*bla* HEK293T cells were plated at 10,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with TO901317 (Cayman chemical #71810) 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 either 1, 1.5, 2, and 3 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 7 – T0901317 dose response with 0, 0.1, 0.5 and 1% DMSO.



LXR alpha-UAS-*bla* HEK293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. TO901317 (Cayman chemical #71810) 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 T0901317 (n=8 for each data point).

References

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3. Millatt, L., Bocher, V., Fruchart, J., Staels, B. (2003) **Liver X receptors and the control of cholesterol homeostasis: potential therapeutic targets for the treatment of atherosclerosis.** *Biochimica et Biophysica Acta* **1631**, 107-118.

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