

# Validation & Assay Performance Summary



## GeneBLAzer<sup>®</sup> TR beta DA Assay Kit

## GeneBLAzer<sup>®</sup> TR beta DA Cells

## GeneBLAzer<sup>®</sup> TR beta-UAS-*bla* HEK 293T Cells

Cat. no. K1389, K1686

### Target Description

The thyroid hormone receptor beta (TR beta) 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<sub>2</sub>-domain (A/B region), a conserved DNA-binding domain (DBD or region C), a linker region (region D), a ligand binding domain (LBD or region E), and in some receptors a variable COOH-terminal (region F) (1). The TR DNA binding domain recognizes and interacts with the thyroid hormone response elements (TREs) (2). TR can bind to this half-site, AGGTCA, as a monomer, a homodimer, or a heterodimer with the RXR (3). The TR/RXR heterodimer is associated with corepressor proteins at the TRE in the absence of ligand (4). Upon binding of thyroid hormone, TR undergoes a conformational change, releasing corepressor proteins and allowing for the interaction with coactivator proteins that enhance TRE-driven gene transcription (5).

Thyroid hormones affect most mammalian tissues. In excess, these hormones may cause weight loss, tachycardia, atrial arrhythmias, and heart failure. TR isoforms have distinct, nonredundant, and tissue-specific functions. TR $\alpha$  is known as an important regulator of heart function. TR beta appears to be a key regulator of hypothalamus-pituitary-thyroid feedback regulation and plasma cholesterol levels (6). Mutations in the gene encoding human TR beta have been associated with general resistance to thyroid hormone (GRTH). This disorder is associated with significant behavioral abnormalities (7).

### Cell Line Description

GeneBLAzer<sup>®</sup> TR beta DA (Division Arrested) cells and TR beta-UAS-*bla* HEK 293T cells contain the ligand-binding domain (LBD) of the human Thyroid hormone receptor alpha (TR beta) fused to the DNA-binding domain of GAL4 stably integrated in the GeneBLAzer<sup>®</sup> UAS-*bla* HEK 293T cell line. GeneBLAzer<sup>®</sup> 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)-TR 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 TR beta DA cells and TR beta-UAS-*bla* HEK 293T cells are functionally validated for Z' and EC<sub>50</sub> concentrations of T3 Thyroid hormone (Figure 1). In addition, TR beta-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, and substrate loading time, and substrate loading 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)

	<u>DA</u>	<u>Dividing</u>
T3 EC <sub>50</sub>	0.3nM	0.2nM
Z'-Factor (EC <sub>100</sub> )	0.70	0.71

Response Ratio	= 20-25
Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 0.5%
Stimulation Time	= 16 hours
Max. [Stimulation]	= 10 nM

### 2. Alternate agonist dose response

T4 EC <sub>50</sub>	= 40 nM
Tetrac EC <sub>50</sub>	= 31 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

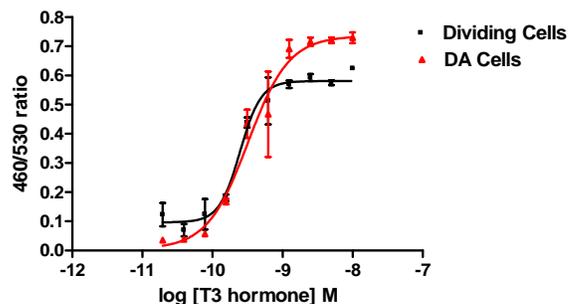
### 6. Assay performance with variable stimulation time

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

### 8. Assay performance with variable DMSO concentration

## Primary Agonist Dose Response

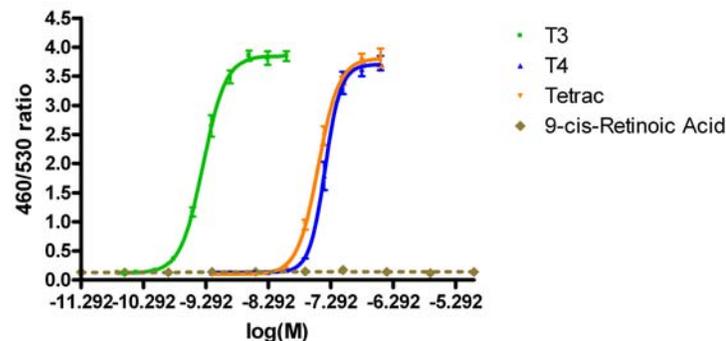
**Figure 1 — TR beta DA and TR beta-UAS-*bla* HEK 293T dose response to T3 Thyroid hormone under optimized conditions**



TR beta DA cells and TR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated in a 384-well format stimulated with a dilution series of T3 Thyroid hormone 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 T3 Thyroid hormone (n=6 for each data point).

## Alternate Agonist Dose Response

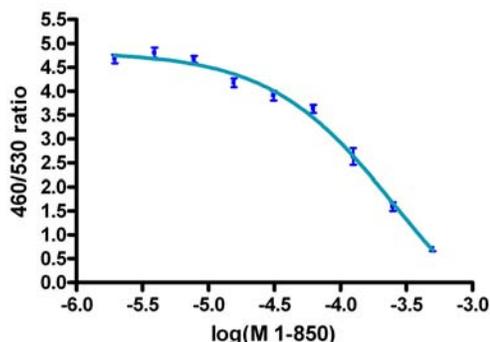
**Figure 2 —T3, T4, Tetrac, and 9-cis-Retinoic Acid Dose Response**



TR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either T3 (Calbiochem #642511), T4-thyroxine (Sigma # T1775), Tetrac (Sigma #T3787), or 9-cis-retinoic acid (Biomol GR-101) 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).

## Antagonist Dose Response

Figure 3 — Thyroid Hormone Receptor Antagonist, 1-850



TR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5%DMSO. Cells were treated with Thyroid Hormone Receptor Antagonist, 1-180(Calbiochem # 609315) and incubated at 37 degrees C for 30 min., followed by 1.2nM T3 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 Ratios are shown plotted against the indicated concentrations of 1-180.

## Dividing Cell Culture and Maintenance

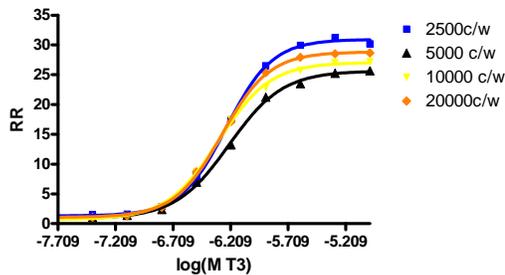
Dividing cells should be maintained at between 5 and 90% confluency in complete growth media and in a humidified incubator at 37°C and 5% CO<sub>2</sub>. Split dividing cells at least twice a week. Do not allow dividing cells to reach confluence.

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 <b>Do not substitute!</b>	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

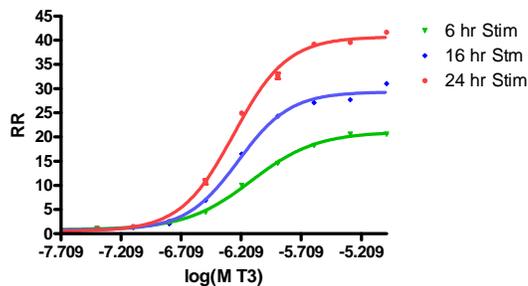
Figure 4— T3 dose response with 2.5, 5, 10, and 20K cells/well



TR beta-UAS-*bla* HEK 293T cells were plated at 2500, 5000, 10,000, or 20,000 or cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with T3 (Calbiochem #642511) for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 $\mu$ M 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 T3 (n=8 for each data point).

## Assay performance with Variable Stimulation Time

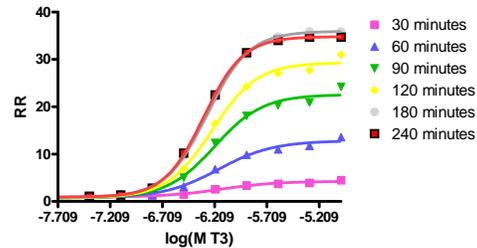
Figure 5 – T3 dose response with 6, 16, and 24 hour stimulation times



TR beta-UAS-*bla* HEK 293T cells (10,000cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate in 0.5% DMSO. T3 (Calbiochem #642511) was then added to the plate over the indicated concentration range for 6, 16, and 24 hours and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate(1 $\mu$ M 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

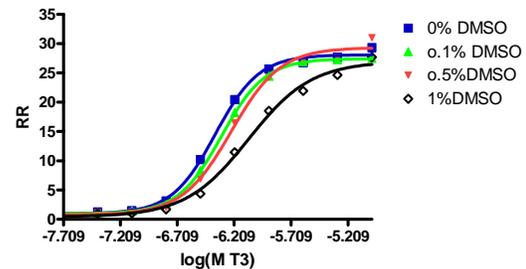
Figure 6 – T3 dose response with 30, 60, 90, 120, 180 and 240 minutes loading times



TR beta-UAS-*bla* HEK 293T cells were plated at 10,000 cells/well in a 384-well format the day of the assay in 0.5%DMSO. Cells were stimulated with T3 (Calbiochem #642511) for 16 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (1 $\mu$ M final concentration of CCF4-AM) for either 30,60, 90, 120, 180, and 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 T3 (n=8 for each data point).

## Assay Performance with variable DMSO concentration

Figure 7 – T3 dose response with 0, 0.1, 0.5 and 1% DMSO.



TR beta-UAS-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. T3 (Calbiochem #642511) 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 $\mu$ M 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 T3 (n=8 for each data point).

## References

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