GLuc-ON™ transcriptional response element (TRE) clones

Catalog# TR100—TR115

User Manual

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USER MANUAL

GLuc-ON™ transcriptional response element (TRE) clones

I. Introduction

GLuc-ON™ transcriptional response element (TRE) clones provide a rapid and sensitive approach for signal pathway analysis in cultured mammalian cells. Using a secreted *Gaussia* Luciferase (GLuc) as the reporter, GeneCopoeia GLuc-ON™ TRE clones are designed for robust and sensitive activation of specific signaling pathways in response to enviromental stimuli or other experimental manipulation. Each GLuc-ON™ TRE clone comes in transfection-ready format, and contains tandem repeats of a TRE placed upstream of a minimal CMV promoter and the GLuc gene. GLuc-ON™ TRE clones offer powerful tools for several applications, including:

- **Drug discovery or validation.** Identify small molecules that either stimulate or inhibit a signaling pathway of interest.
- **Analyze response of cells to proteins and peptides.**
- **Analyze response of cells to hormones.**
- **Functional genomics.** Analyze the biological effects on a signaling pathway in response to gene activation, overexpression, knockdown, knockout, or mutagenesis.
- **Viral infection.** Analyze response of cells to viruses.

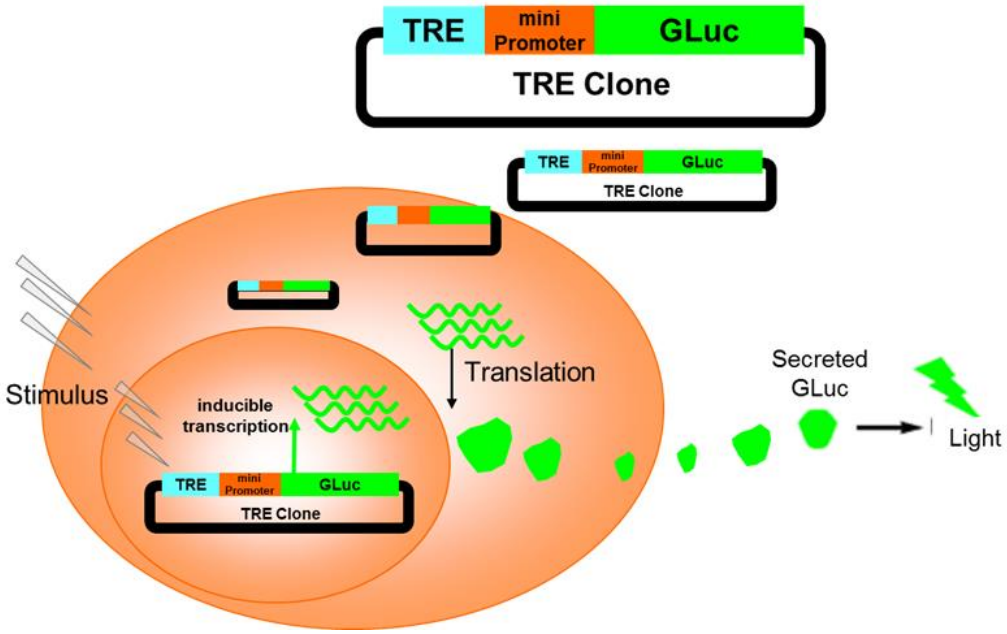

Figure 1. How GLuc-ON™ transcriptional response element (TRE) clones work.

Advantages

Live cell assays
- Naturally secreted GLuc reporter
- No lysis of the cells is necessary
- Save samples, reduce variation, and simplify experiments.

Real-time study
- Data is generated quickly
- Closely resembles real-time activities

High-throughput compatible
- High sample number compatible

High sensitivity
- GLuc is 1,000-fold more sensitive than firefly or *Renilla* luciferases

- **Convenience**
  - All TRE clones are transfection-ready

The GLuc-ON™ TRE response elements are engineered to provide strong activation of GLuc expression with low background. The GLuc reporter is a secreted protein, permitting convenient and rapid detection in the cell culture medium without lysing the cells (Figure 2).

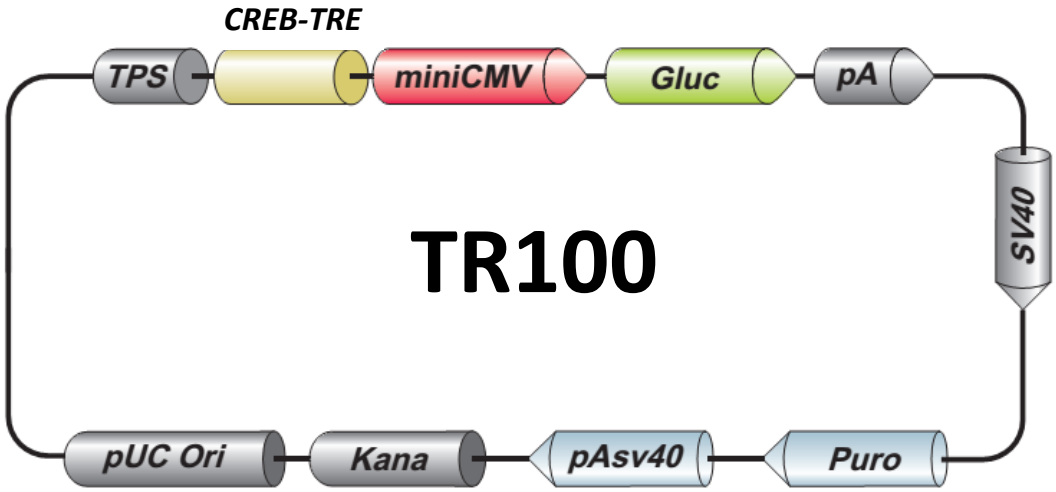

Figure 2. GLuc-ON™ CREB Transcriptional Response Element (TRE) Clone (TR100) as an example of GLuc-ON™ TRE clone structure.

II. List of TRE clones

Catalog#	Product	Concentration	Volume
Control clones			
TR001	DNA-based transcriptional response element(TRE) negative control clone	500ng/μL	40μL
TRE clones			
TR100	CREB TRE clone for cAMP/PKA signaling pathway	500ng/μL	40μL
TR101	NFAT TRE clone for PKC/Ca++ signaling pathway	500ng/μL	40μL
TR102	TCF/LEF TRE clone for Wnt signaling pathway	500ng/μL	40μL
TR103	Oct4 TRE clone for Oct4 signaling pathway	500ng/μL	40μL
TR104	HNF4 TRE clone for HNF4 signaling pathway	500ng/μL	40μL
TR105	JNK TRE clone for SAPK/JNK signaling pathway	500ng/μL	40μL
TR106	PKC TRE clone for PKC/MAPK signaling pathway	500ng/μL	40μL
TR107	EGR1 TRE clone for EGR1 pathway	500ng/μL	40μL
TR109	MEF2 TRE clone for MEF2 signaling pathway	500ng/μL	40μL
TR110	ERSE TRE clone for ER stress pathway	500ng/μL	40μL
TR111	GR TRE clone for glucocorticoid receptor signaling pathway	500ng/μL	40μL
TR112	PPAR TRE clone for peroxisome proliferator-activated receptor signaling pathway	500ng/μL	40μL

Continued next page

II. List of TRE clones (continued)

Catalog#	Product	Concentration	Volume
TRE clones			
TR113	PR TRE clone for progesterone receptor signaling pathway	500ng/μL	40μL
TR114	LXRa TRE clone for liver X receptor signaling pathway	500ng/μL	40μL
TR115	NFκB TRE clone for NFκB - regulated signaling pathway	500ng/μL	40μL

* The GLuc-ON™ TRE clones are all endotoxin-free and transfection-ready. They will be shipped at room temperature and should be stored in -20℃. They will be stable for at least 12 months at -20℃.

III. Materials required but not supplied

- PBS (Corning, 21-040-CVR)
- Trypsin-EDTA (Corning, 25-053-CI)
- DMEM (Corning, 10-013-CVR)
- Fetal bovine serum (MP, 2916754)
- Opti-MEM® I Reduced Serum Medium (Gibco, 31985-070)
- EndoFectin™-Lenti (GeneCopoeia, EFL1001-01/EFL1001-02)
  You may use other transfection reagents from GeneCopoeia, e.g. EndoFectin™-Max (GeneCopoeia, EFM1004-01/EFM1004-02), EndoFectin™-HepG2 (GeneCopoeia, EF005/EF006) .
- Secrete-Pair™ Luciferase Assay System
  Secrete Pair™ *Gaussia* Luciferase Assay Kit (GeneCopoeia, LF061/LF062)
- 96-Well Cell Culture Plate (corning, 3599)
- 100 × 20 mm Tissue Culture Dish (BD Falcon, 353003)
- 96-well Assay Plate(corning, 3340)
- Luminometer (TECAN, Infinite F200 PRO)

IV. Experimental Procedures

Protocol 1: Co-transfected TRE-reporter and Plasmid stimulator

A. Experimental transfections

Day 1: Plate Cells

1. Grow HEK 293 cells in DMEM/FBS medium to approximately 90% confluence.

2. Harvest cells via trypsinization. Remove the medium, wash the cells with PBS and add the trypsin. After 2 minutes, add 3 × volume of medium to terminate the digestion reaction. Collect the cell suspension and pellet the cells by centrifugation.

3. Aspirate the supernatant and resuspend the viable cells at a concentration of 5 × 10^4/mL.

4. Dispense 200μL cells/well suspension into a 96-well plate (perform each test condition in triplicate).

5. Place the plate in a tissue culture incubator at 37° C for 24 hours.

Day 2: Co-transfected TRE-reporter and plasmid stimulator

Each well of 96-well plate to be transfected requires 40μL Opti-MEM, 0.6μL EndoFectin™-Lenti, 100ng TRE-Reporter and 100ng plasmid stimulator.

Well	TRE Clone	Negative control (TR001)	Plasmid stimulator	Plasmid negative stimulator	Opti-MEM	Endo Fectin Lenti	Opti-MEM	Total volume
i	100ng (1.0μL)		100ng (1.0μL)		20μL	0.3μL	20μL	41.3μL
ii	100ng (1.0μL)			100ng (1.0μL)	20μL	0.3μL	20μL	41.3μL
iii		100ng (1.0μL)	100ng (1.0μL)		20μL	0.3μL	20μL	41.3μL
iv		100ng (1.0μL)		100ng (1.0μL)	20μL	0.3μL	20μL	41.3μL

Note: See the following procedure for detailed instruction of preparing transfection system.

Experimental Transfection:

i. TRE Reporter + Plasmid stimulator

Control Transfections:

ii.TRE Reporter + Plasmid- negative stimulator
iii. TRE negative control+ Plasmid stimulator
iv. TRE negative control + Plasmid- negative stimulator

1. For each well,use 20μL Opti-MEM® I Medium to dilute 0.6μL EndoFectin™-Lenti Transfection Reagent and use another 20μL Opti-MEM® I Medium to dilute 100ng TRE-Reporter and 100ng Plasmid stimulator.

2. Add diluted EndoFectin™-Lenti Transfection Reagent to the tube of diluted DNA, Mix gently .

3. Incubate at room temperature for 15-25 minutes.

4. Use the pipetter to remove 42.6μL culture medium from the 96-well plate, then add the DNA-EndoFectin™-Lenti Transfection Reagent complexes dropwise to each well (total 42.6μL). Mix gently .

5. Incubate the cells in a tissue culture incubator for 24-48 hours.

Day 4: Collect the medium

After 24- 40 hours of transfection, collect the medium to prepare the luciferase assay.

B. Luciferase assay

1. Thaw the cultured cells and Buffer GL-S (10×) thoroughly at room temperature, inverting the tube several times, and then vortex for 3-5 sec. Dilute 1:10 in distilled water to make 1× Buffer GL-S. Prepare 100μL of 1 × Buffer GL-S for each reaction (well). Duplicates or triplicates for each sample are recommended.

2. Prepare the GLuc Assay Working Solution (e.g.10 samples) by adding 10μL of Substrate GL to 1 mL of 1 × Buffer GL-S. Mix well by inverting the tube several times.

3. Incubate at room temperature for 25 minutes (capped and protected from light) before adding to the samples.

4. Set up the luminometer. Set the measurement for 1–3 seconds of integration.

5. Pipet culture medium samples (10µL/well, in duplicates or in triplicates) into a 96-well assay plate.

6. Add the GLuc Assay Working Solution from Step 3 (100µL/ well or tube) to the samples from Step 5. Gently tap the plate several times to mix the sample and substrate.

7. Analyze luciferase activity .

Note: This protocol is for enhanced signal stability using GL-S buffer.

Protocol 2: Transfected TRE-reporter with Small Molecule/ Organic Compound

A. Experimental transfections

Day 1: Plate Cells

1. Grow HEK 293 cells in DMEM/FBS medium to approximately 90% confluence.

2. Harvest cells via trypsinization. Remove the medium, wash the cells with PBS and add the trypsin. After 2 minutes, add 3× volume of medium to terminate the digestion reaction. Collect the cell suspension and pellet the cells by centrifugation.

3. Aspirate the supernatant and resuspend the viable cells at a concentration of 1 × 10^5/mL.

4. Dispense 100µL cells/well suspension into a 96-well plate (perform each test condition in triplicate).

5. Place the plate in a tissue culture incubator at 37° C for 24 hours.

Day 2: Transfected TRE-reporter with Small Molecule/ Organic Compound

Each well of 96-well plate to be transfected requires 40µL opti-MEM, 0.3µL EndoFectin™-Lenti, 100ng TRE-Reporter .

Well	TRE Clone	Negative control (TR001)	Small Molecule/	Opti-MEM	Endo Fectin-Lenti	Opti-MEM	Total volume
i	100ng (1.0μL)		0	20μL	0.3μL	20μL	41.3μL
ii	100ng (1.0μL)		1×	20μL	0.3μL	20μL	41.3μL
iii	100ng (1.0μL)		10×	20μL	0.3μL	20μL	41.3μL
iv		100ng (1.0μL)	0	20μL	0.3μL	20μL	41.3μL
v		100ng (1.0μL)	1×	20μL	0.3μL	20μL	41.3μL
vi		100ng (1.0μL)	10×	20μL	0.3μL	20μL	41.3μL

Note: 1× is a lowest amount of small molecule/organic compound expected to generate a response. See the following procedure for detailed instructions for preparing transfection system.

Experimental transfections:

i. TRE Reporter + 0 Small Molecule/Organic Compound
ii.TRE Reporter + 1 × Small Molecule/Organic Compound
iii. TRE Reporter + 10 × Small Molecule/Organic Compound

Control transfections:

iv. TRE negative control + 0 Small Molecule/Organic Compound
v. TRE negative control + 1 × Small Molecule/Organic Compound
vi. TRE negative control + 10 × Small Molecule/Organic Compound

1. For each well, use 20μL Opti-MEM® I Medium to dilute 0.3μL EndoFectin™-Lenti Transfection Reagent, and use another 20μL Opti-MEM® I Medium to dilute 100ng TRE-Reporter.
2. Add diluted EndoFectin™-Lenti Transfection Reagent to the tube of diluted DNA, Mix gently .
3. Incubate at room temperature for 15-25 minutes.
4. Add the DNA-EndoFectin™-Lenti Transfection reagent complexes dropwise to each well, Mix gently .
5. Incubate the cells in a tissue culture incubator for 24 hours.

Day 3: Add Small Molecules/Organic Compounds

1. Dilute the stock solution (Small Molecule/ Organic Compound) to 10× and 1× in DMEM/FBS medium .

1. Remove the old medium, and add 200µL 10× induction medium or 1× induction medium to the cells .

2. Return the plate to the tissue culture incubator and induce for 6-24 hours.

Day 4: Collect the medium

After 6-24 hours of stimulation, collect the medium and prepare the luciferase assay.

B. Luciferase assay

1. Thaw the cultured cells and Buffer GL-S (10×) thoroughly at room temperature, inverting the tube several times, and then vortex for 3-5 sec. Dilute 1:10 in distilled water to make 1 × Buffer GL-S. Prepare 100ul of 1 x Buffer GL-S for each reaction (well). Duplicates or triplicates for each sample are recommended.

2. Prepare the GLuc Assay Working Solution (e.g.10 samples) by adding 10µL of Substrate GL to 1 mL of 1 × Buffer GL-S. Mix well by inverting the tube several times.

3. Incubate at room temperature for 25 minutes (capped and protected from light) before adding to the samples.

4. Set up the luminometer. Set the measurement for 1–3 seconds of integration.

5. Pipet culture medium samples (10µL/well, in duplicates or in triplicates) into a 96-well Assay Plate.

6. Add the GLuc Assay Working Solution from Step 3 (100µL/ well or tube) to the samples from Step 5. Gently tap the plate several times to mix the sample and substrate.

7. Analyze luciferase activity .

Note: This protocol is for enhanced signal stability using GL-S buffer.

Protocol 3: Co-Transfected TRE-reporter with Chemical/Protein/AD-Virus Stimulus

A. Experimental transfections

Day 1: Plate Cells

1. Grow HEK 293 cells in DMEM/FBS medium to approximately 90% confluency.
2. Harvest cells via trypsinization. Remove the medium, wash the cells with PBS, and add the trypsin. After 2 minutes, add 3× volume of medium to terminate the digestion reaction. Collect the cell suspension and pellet the cells by centrifugation.
3. Aspirate the supernatant and resuspend the viable cells at a concentration of 1 × 10^5/ml.
4. Dispense 100µL cells/well suspension into a 96-well plate (perform each test condition in triplicate).
5. Place the plate in a tissue culture incubator at 37° C for 24 hours.

Day 2: Co-Transfected TRE-reporter with Chemical/Protein/AD-Virus Stimulus

Each well of 96-well plate to be transfected requires 40µL opti-MEM, 0.3µL EndoFectin™-Lenti,100ng TRE-Reporter .

Well	TRE-Reporter	Chemical/ Protein	AD-Virus Stimulus	Opti-MEM	Endo Fectin-Lenti	Opti-MEM	Total volume
i	100ng (1.0µL)	0	TRE AD-Virus(10MOI)	20µL	0.3µL	20µL	41.3µL
	100ng (1.0µL)	0	TRE AD-Virus(100MOI)	20µL	0.3µL	20µL	41.3µL
ii	100ng (1.0µL)	0	TRE AD-Virus(10MOI)	20µL	0.3µL	20µL	41.3µL
	100ng (1.0µL)	0	TRE AD-Virus(100MOI)	20µL	0.3µL	20µL	41.3µL
iii	100ng (1.0µL)	1×	TRE AD-Virus(10MOI)	20µL	0.3µL	20µL	41.3µL
	100ng (1.0µL)	1×	TRE AD-Virus(100MOI)	20µL	0.3µL	20µL	41.3µL
iv	100ng (1.0µL)	1×	TRE AD-Virus(10MOI)	20µL	0.3µL	20µL	41.3µL
	100ng (1.0µL)	1×	TRE AD-Virus(100MOI)	20µL	0.3µL	20µL	41.3µL

Note: See the following procedure for detailed instructions for preparing transfection system.

Experimental transfections:

i. TRE Reporter + 0 Chemical/Protein + TRE AD-Virus (10MOI/100MOI)
iii. TRE Reporter + 1 × Chemical/Protein + TRE AD-Virus (10MOI/100MOI)

Control transfections:

ii. TRE Reporter + 0 Chemical/Protein +TRE AD-GFP control (10MOI/10MOI)
iv.TRE Reporter + 1 × Chemical/Protein + TRE AD-GFP control (10MOI/10MOI)

1. For each well, use 20μL Opti-MEM® I Medium to dilute 0.3μL EndoFectin™-Lenti Transfection Reagent, and use another 20μL Opti-MEM® I Medium to dilute 100ng TRE-Reporter.

2. Add diluted EndoFectin™-Lenti Transfection Reagent to the tube of diluted DNA. Mix gently .

3. Incubate at room temperature for 15-25 minutes.

4. Add the DNA-EndoFectin™-Lenti Transfection reagents complexes dropwise to each well. Mix gently .

5. Incubate the cells in a tissue culture incubator for 24 hours.

Day 3: Add Chemical,Protein,AD-Virus Stimulus

1. Dilute the stock solution (with Chemical,Protein,AD-Virus Stimulus) in DMEM/FBS medium .

2. Remove the old medium, and add 200μL induction medium to the cells .

3. Return the plate to the tissue culture incubator and induce for 6-24 hours.

Day 4: Collect the medium

After 6-24 hours of stimulation, collect the medium and prepare the luciferase assay.

B. Luciferase assay

1. Thaw the cultured cells and Buffer GL-S (10×) thoroughly at room temperature, inverting the tube several times, and then vortex for 3-5 sec. Dilute 1:10 in distilled water to make 1 × Buffer GL-S. Prepare 100ul of 1 × Buffer GL-S for each reaction (well). Duplicates or triplicates for each sample are recommended.

2. Prepare the GLuc Assay Working Solution (e.g.10 samples) by adding 10µL of Substrate GL to 1 mL of 1 × Buffer GL-S. Mix well by inverting the tube several times.

3. Incubate at room temperature for 25 minutes (capped and protected from light) before adding to the samples.

4. Set up the luminometer. Set the measurement for 1–3 seconds of integration.

5. Pipet culture medium samples (10µL/well, in duplicates or in triplicates) into a 96-well Assay Plate.

6. Add the GLuc Assay Working Solution from Step 3 (100µL/ well or tube) to the samples from Step 5. Gently tap the plate several times to mix the sample and substrate.

7. Analyze luciferase activity .

Note: This protocol is for enhanced signal stability using GL-S buffer.

V. Limited Use License and Warranty

Limited use license

The following terms and conditions apply to use of the GLuc-ON™ transcriptional response element (TRE) clones. If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. Use of any part of the Product constitutes acceptance of the above terms.

Limited warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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