GLuc-ON™ transcriptional response element (TRE) lentiviral clones

Catalog# TR200-TR207

User Manual

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USER MANUAL

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I. Introduction

GeneCopoeia's GLuc-ON™ transcriptional response element (TRE) lentiviral clones enable rapid and sensitive signal pathway analysis in mammalian cell lines. Using a secreted *Gaussia* Luciferase (GLuc) as the reporter, GeneCopoeia GLuc-ON™ TRE lentiviral clones are designed for robust and sensitive activation of specific signaling pathways in response to environmental stimuli or other experimental manipulation. Each GLuc-ON™ TRE lentiviral clone contains tandem repeats of a TRE placed upstream of a minimal CMV promoter and the Gluc gene.. GLuc-ON™ TRE clones are 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.

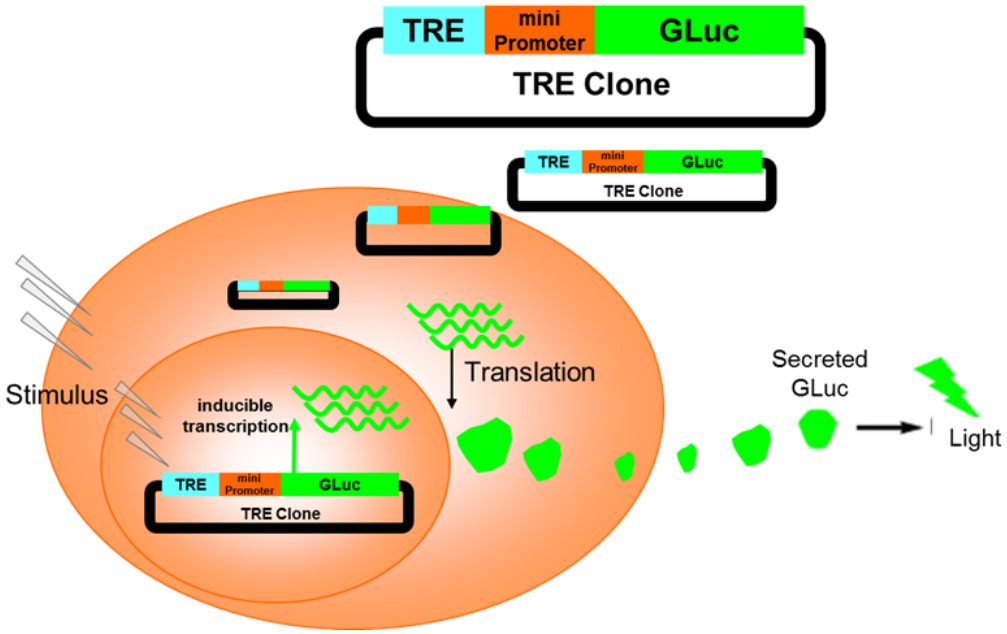

Figure 1. How GLuc-ON™ transcriptional response element (TRE) lentiviral clones work.

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 1000-fold more sensitive than firefly or *Renilla* luciferase

Convenience
- All Gluc-ON™ TRE lentiviral clones are ready for transfection into lentiviral packaging cell lines

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).

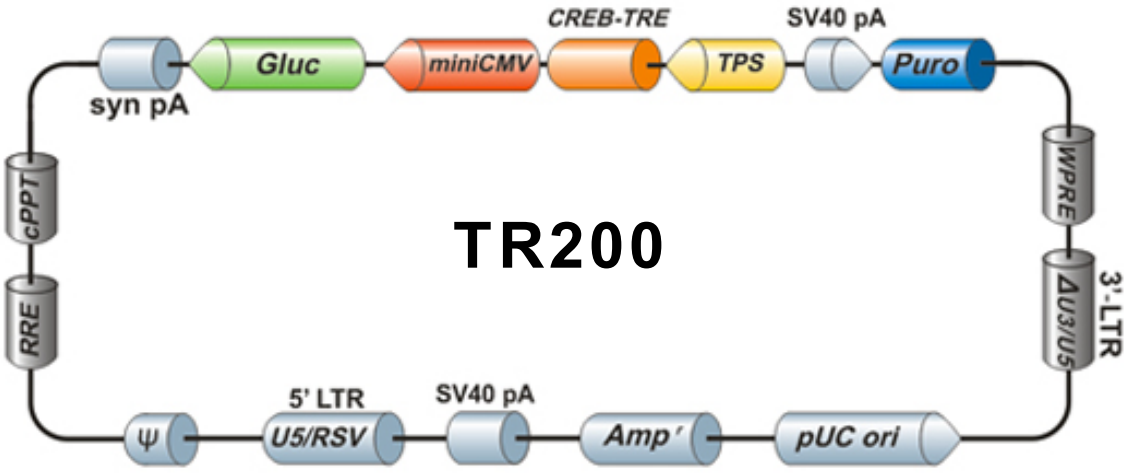

Figure 2. Example of a GLuc-ON™ TRE lentiviral clone

II. List of TRE clones

Catalog#	Product	Concentration	Volumn
Control clone			
TR002	DNA-based transcriptional response element(TRE) negative control lentiviral clone	500ng/μL	40μL
TRE clones			
TR200	CREB TRE lentiviral clone for cAMP/PKA signaling pathway	500ng/μL	40μL
TR201	NFAT TRE lentiviral clone for PKC/Ca++ signaling pathway	500ng/μL	40μL
TR202	TCF/LEF TRE lentiviral clone for Wnt signaling pathway	500ng/μL	40μL
TR206	PKC TRE lentiviral clone for PKC/MAPK signaling pathway	500ng/μL	40μL
TR207	EGR1 lentiviral TRE clone for EGR1 pathway	500ng/μL	40μL

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

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, Z01020A)
  You may use other transfection reagent from GeneCopoeia : EndoFectin™-CHO (GeneCopoeia, Z01030A) ;EndoFectin™-Plus(GeneCopoeia, Z01010A) .
- Secrete-Pair™ Luciferase Assay System
  Secrete Pair™ Gaussia Luciferase Assay Kit(GeneCopoeia, SPGA-G010)
  The Secrete Pair Gaussia Luciferase Assay Kit is available for Gaussia luciferase assay alone as well.
- 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）

Protocol: Transduced TRE-reporter lentivirus with small molecule/organic compound

The following procedure begins after the production of TRE lentiviral particles. GeneCopoeia recommends our Lenti-Pac™ Lentivral Packaging System (http://www.genecopoeia.com/product/lentiviral-packaging-kit-cells/) for viral particle production.

A. Experimental transductions

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 termination 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 500µL cells/well suspension into a 24-well plate (each test condition perform in triplicate).
5. Place the plate in a tissue culture incubator at 37° C for 24 hours.

Experimental transductions:

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 transductions :

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

Day 2: Transduce cells with TRE-reporter lentiviral particles

1. Add sufficient viral particles to achieve the optimal MOI for your cell line.
2. Place the plate at 4° C for 2 hours.
3. Transfer the plate to a tissue culture incubator at 37° C for 48 hours.

Day 4: Transduce cells with TRE-reporter lentiviral particles

1. 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 termination the digestion reaction. Collect the cell suspension and pellet the cells by centrifugation.
2. Aspirate the supernatant and transfer the cells to 1 well of a 6-well plate in medium containing puromycin.
3. Transfer the plate to a tissue culture incubator at 37° C for 6 days.

Day 10: Prepare cells for chemical stimulation

1. Remove the medium and harvest the cells via trypsinization.
2. Resuspend the viable cells at a concentration of 1x 10^5/ml, Dispense 100ul suspension into a 96-well plate (each test condition perform in triplicate).
3. Place the plate in a tissue culture incubator at 37° C for 24 hours.

Day 11: Add Small Molecules/Organic Compounds

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

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

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

Day 12: Collect the medium

After 6-24 hours of stimulation,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 100ul of 1xBuffer 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 protect 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) lentiviral clones (the Product). 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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