



## pTF-Luc Reporter Vectors

Catalog Number LR-2XXX

(For Research Use Only)

### Introduction

pTF-Luc reporter vectors are a series of firefly luciferase-based reporter constructs for quantitative measurement of the activities of transcription factors (TFs) in cells. Each vector contains a cis-element (DNA binding sequence), a minimal promoter, and firefly luciferase gene. When a TF is activated, it binds to cis-element and results in induction of luciferase gene expression. Therefore, luciferase activity represents the activity of the TF.

### Recommend transfection and assay

We recommend using FuGENE 6 (Roche) for the transfection of pTF-Luc reporter vectors. For difficult-to-transfect cell type such as primary cells, we recommend using Fugene HD (Roche) for the transfection. The transfection can be done in 6-well or 12 well plates.

The following protocol is designed for adherent cultures in **6-well** plates using FuGENE 6. If you use a different size of plate or flasks, adjust the components in proportion to the surface area of your container.

1. Plate  $1-4 \times 10^5$  cells in 2 ml of growth medium containing serum without antibiotics in a 6-well culture plate at one day before transfection, which will yield 50-80% confluence on the day of transfection.
2. For each transfection, dilute 0.5-1  $\mu\text{g}$  of the reporter vector with 100  $\mu\text{l}$  of serum-free culture medium, and in a separate tube, dilute 3  $\mu\text{l}$  FuGENE 6 Reagent with 100  $\mu\text{l}$  of serum-free culture medium (add transfection reagent directly into the medium and don't touch the wall of the tube). Add the diluted reporter vector to the diluted transfection reagent and gently mix. Incubate for 15-30 min at room temperature. Once the FuGENE 6 Reagent is diluted, it needs to use within 45 min.
3. Add 200  $\mu\text{l}$  of DNA/FuGENE complex to on the cells in a drop-wise manner. Evenly distribute the complex by gently rocking the plate back and forth. Incubate the cells at 37°C in a CO<sub>2</sub> incubator for overnight.
4. If the starvation is required, replace the medium with serum free or low serum medium (0.2% serum) for 6 -16 hours, and treat the cell with the selected stimulus for 8-14 hours.

5. Alternatively, to study the effects of a gene of interest, cotransfect each pTF-Luc with a gene expression vector of interest.

6. Lyse the attached cells by adding lysis buffer (Firefly Luciferase Lysis Buffer 5x CAT#LS-001, Signosis) to each well. Use approximately 200  $\mu\text{l}$  per well for a 6-well plate. To detach cells from the plate, freeze and thaw the plate once and pipette the mixture up and down. Transfer the cell

lysate/buffer solution to a clean 1.5-ml microcentrifuge tube, which is ready for luciferase assay or store at -80°C for the future use. Assay for luciferase activity following the instructions given by the supplier (Firefly Luciferase Substrate CAT#LUC015 or LUC100, Signosis). Refer to step 6 in the Firefly Luciferase Substrate manual. Alternatively, the relevant information can be found below to proceed with the assay.

7. Thaw luciferase substrate at room temperature prior to use. Perform the assay when the substrate reaches room temperature.

NOTE: Do not thaw the substrate at temperature above 30°C.

8. Add 50  $\mu\text{l}$  of luciferase substrate to each well and gently pipette up and down.

9. Immediately read the plate in a luminometer, with setting at 10 seconds integration.

### E. coli transform to propagate the plasmids

1. Transform *E. coli* competent cells with the plasmid.
2. Plate the transformed cells on LB plates containing 100  $\mu\text{g}/\text{mL}$  Ampicillin and grow overnight at 37°C.
3. Transfer a single colony to 1-2 ml LB medium containing 100  $\mu\text{g}/\text{mL}$  Ampicillin and shake at 37°C overnight.
4. Prepare plasmids and check on gel.

## Diagram of pTF-Luc reporter vectors

