Certificate of Analysis

pGL4.33[*luc2P*/SRE/Hygro] Vector

Part No.	Size
E134A	20µg

Part# 9PIE134

Revised 10/16

Instructions for use of this product can be found in the pGL4 Luciferase Reporter Vectors Technical Manual #TM259, available online at: **www.promega.com/protocols/**

Description: The pGL4.33[*luc2P*/SRE/Hygro] Vector[a–c] contains a Serum Response Element (SRE) that drives transcription of the luciferase reporter gene *luc2P* in response to activation of MAPK/ERK signaling pathway. *luc2P* is a synthetically derived luciferase sequence with humanized codon optimization. The *luc2P* gene also contains hPEST, a protein destabilization sequence. The protein encoded by *luc2P* responds more quickly to induction than the protein encoded by the *luc2* gene. The vector backbone contains an ampicillin resistance gene to allow selection in *E. coli* and the mammalian selectable marker for hygromycin resistance.

Concentration: 1µg/µl.

GenBank® Accession Number: FJ773212.

Storage Buffer: The pGL4.33[*luc2P*/SRE/Hygro] Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

Storage Conditions: See the Product Information Label for storage temperature recommendations and expiration date.

Usage Note: Mix well prior to use.

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Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acid Assay: RNA, single-stranded DNA and chromosomal DNA are not evident in a specified sample of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of this vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: www.promega.com/vectors

Restriction Digestion: The functional purity of this vector DNA is verified by complete digestion with selected restriction enzymes at 37°C for 1 hour. Samples are examined by agarose gel electrophoresis, and cut and uncut vector DNA are compared with marker DNA.

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE.

Researchers shall have no right to modify or otherwise create variations of the nucleotide sequence of the luciferase gene except that researchers may (1) create fused gene sequences, and (2) insert and remove nucleic acid sequences in splicing research. No other use or transfer of this product or derivatives is authorized. Researchers must either (1) use luminescent assay reagents purchased from Promega for all determinations of luminescence activity of this product and its derivatives; or (2) contact Promega to obtain a license for use of the product. For any uses outside this label license, contact Promega for supply and licensing information. This product is for research use only; no commercial use is allowed. For a full copy of this label license, including the definition of "commercial use," go to: **www.promega.com/LULL**

[b]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

[c]U.S. Pat. No. 7,728,118.

Signed by:

R. Wheeler, Quality Assurance

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Features List and Map for the pGL4.33[*luc2P*/SRE/Hygro] Vector

SRE	33–147
minimal promoter (minP)	180–210
luc2P reporter gene	243–2018
SV40 late poly(A) region	2058–2279
SV40 early enhancer/promoter	2327–2745
synthetic hygromycin (Hyg^r) coding region	2770–3807
synthetic poly(A) signal	3831–3879
reporter vector primer 4 (RVprimer4) binding region	3946–3965
ColE1-derived plasmid replication origin	4203
synthetic β-lactamase (Amp^r) coding region	4994–5854
synthetic poly(A) signal/transcriptional pause site	5959–6112
reporter vector primer 3 (RVprimer3) binding region	6061–6080

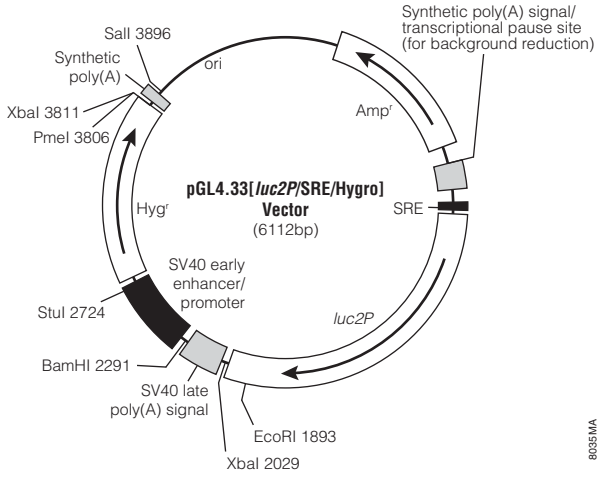

Figure 1. pGL4.33[*luc2P*/SRE/Hygro] Vector map.

Sequence information and restriction enzyme tables for the pGL4 Vectors are available online at: **www.promega.com/vectors/**

Additional information is available in Technical Manual #TM259, available online at: **www.promega.com/protocols/**

Sample Protocol to Determine Induction of Luciferase by FBS + PMA in HEK293 Cells Transfected with the pGL4.33[*luc2P*/SRE/Hygro] Vector

Materials to Be Supplied by the User

- Dulbecco's PBS (DPBS)
- 0.05% (w/v) trypsin in DPBS
- DMEM
- DMEM supplemented with 0.5%, 10% and 40% fetal bovine serum (DMEM/FBS)
- Phorbol 12-myristate 13-acetate (PMA, Promega Cat.# V1171 or Sigma Cat.# P8139), 1mg/ml solution in DMSO
- ONE-Glo™ Luciferase Assay System (Cat.# E6110)
- HEK293 cells
- transfection reagent

Day 1: Plate Cells

1. Grow HEK293 cells in DMEM/FBS to approximately 75% confluency.
2. Harvest cells via trypsinization: Remove the DMEM/FBS, wash the cells with DPBS and add the trypsin/DPBS (1X volume). After 2 minutes, add a 4X volume of DMEM/FBS, collect the cell suspension and pellet the cells by centrifugation. Aspirate the supernatant, and resuspend in DMEM/FBS. We have routinely used a concentration of 10,000–15,000 viable cells/100µl DMEM/FBS.
3. Dispense 100µl of the cell suspension into the wells of a 96-well plate. Plate enough wells to perform each test condition in triplicate.
4. Cover the plate, and place it in a tissue culture incubator at 37°C overnight (or for 24 hours).

Day 2: Transfect Cells

1. Transfect the cells using a high-efficiency transfection reagent. Each well of cells in a 96-well plate requires 0.1µg pGL4.33[*luc2P*/SRE/Hygro] Vector DNA. Transfection conditions may require optimization.
2. Cover the plate, and place it in a tissue culture incubator at 37°C.
3. After 4–6 hours, change the medium to DMEM/0.5%FBS (100µl per well) to start serum starvation.

Day 3: Induce Transfected Cells

1. Prepare 2X induction and 2X control solutions. Calculate the volume of 2X induction and 2X control solution by multiplying the number of wells needed for each solution by 50µl, and prepare 110% of this amount.
   - 2X induction solution: 40%FBS plus 20ng/ml PMA in DMEM
   - 2X control solution: DMEM
2. Remove 50µl of medium from wells that will be treated with either 2X induction solution or 2X control solution.
3. Add 50µl of 2X induction solution to the cells to be induced and 50µl of 2X control solution to the control noninduced cells.
4. Return the plate to the tissue culture incubator, and induce for 6 hours.
5. Analyze luciferase activity using an appropriate luciferase detection assay. We have observed comparable results for fold induction of the vector using a variety of luciferase reagents, including: Bright-Glo™ Luciferase Assay System (Cat.# E2610, Technical Manual #TM052); ONE-Glo™ Luciferase Assay System (Cat.# E6110, Technical Manual #TM292); Dual-Luciferase® Reporter Assay System (Cat.# E1910, Technical Manual #TM040); and Dual-Glo® Luciferase Assay System (Cat.# E2920, Technical Manual #TM058).
6. Calculate the fold induction as follows:

$$\text{fold induction} = \frac{\text{average relative light units of induced cells}}{\text{average relative light units of control cells}}$$

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