Certificate of Analysis

pGL4.47[*luc2P*/SIE/Hygro] Vector:

Part No.	Size
E404A	20µg

Part# 9PIE404
Revised 10/16

Description: The pGL4.47[*luc2P*/SIE/Hygro] Vector[a–c] contains five copies of the sis-inducible element (SIE) that drives transcription of the luciferase reporter gene *luc2P* (*Photinus pyralis*). *luc2P* is a synthetically derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription. The *luc2P* gene contains hPEST, a protein destabilization sequence, which allows luc2P protein levels to respond more quickly than those of luc2 to induction of transcription. The vector backbone contains an ampicillin resistance gene to allow selection in *E. coli* and a gene for hygromycin resistance to allow selection of stably transfected mammalian cell lines.

Concentration: 1µg/µl.

GenBank® Accession Number: JQ858512.

Storage Buffer: The GL4.47[*luc2P*/SIE/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See the product information label for storage temperature recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the product information label.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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Quality Control Assays

Nuclease Assay: Following incubation of 1µg of the 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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Sequence: The pGL4.47[*luc2P*/SIE/Hygro]Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors/**

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[b]U.S. Pat. No. 7,728,118.
[c]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

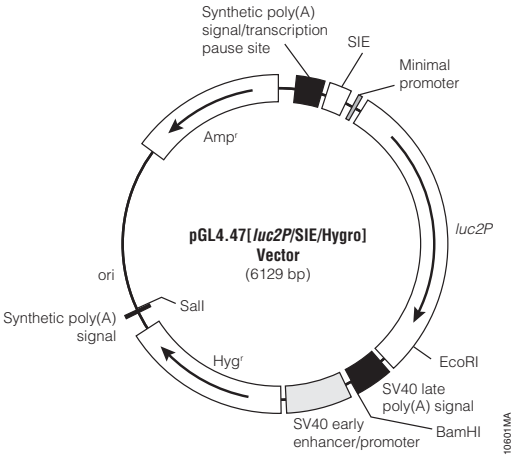
Signed by:

R. Wheeler, Quality Assurance

Promega

pGL4.47[*luc2P*/SIE/Hygro] Vector Features List and Map:

SIE response element	285–409
Minimal promoter	455–485
luc2P reporter gene	518–2293
SV40 late poly(A) signal	2333–2554
SV40 early enhancer/promoter	2602–3020
Synthetic hygromycin (Hyg^r) coding region	3045–4082
*Col*E1-derived plasmid replication origin	4478
Synthetic β-lactamase (Amp^r) coding region	5269–6129
Synthetic poly(A) signal sequence	4106–4154
Synthetic poly(A) signal/transcriptional pause site	105–258
Reporter Vector primer 3 (RVprimer3) binding region	207–226
Reporter Vector primer 4 (RVprimer4) binding region	4221–4240



Sequence information for the pGL4 Vectors is available online at:
www.promega.com/vectors/

Example Protocol

In this example protocol, the pGL4.47[*luc2P*/SIE/Hygro] vector is used to measure activation of the SIE in HEK293 cells upon treatment with interleukin 6. In designing such experiments, it is important that the chosen cell type can be transfected efficiently and that it expresses the proper components of the signaling pathway of interest in order to generate the biological response. Protocol optimization may be required for your particular cell type and assay conditions.

Materials to be Supplied by User

- DMEM (Life Technologies Cat.# 11995)
- FBS (HyClone Cat.# SH30070.03)
- Dulbecco's PBS (DPBS; Life Technologies Cat. # 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- Human recombinant interleukin 6 (IL-6, Life Technologies Cat.# PHC0061)
- DMSO (Sigma Cat.# D2650)
- ONE-Glo™ Luciferase Assay System (Cat.# E6120)
- HEK293 cells

Day 1: Plate Cells

1. Plate 10ml of HEK293 cells at 2×10^5 cells/ml in a 10cm dish in complete medium (DMEM + 10% FBS).
2. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Transfection

1. Dilute 10µg pGL4.47[*luc2P*/SIE/Hygro] Vector DNA in 500µl Opti-MEM® I.
2. Add 30µl FuGENE® HD to a 3:1 lipid:DNA ratio and mix. Incubate at room temperature for 15 minutes.
3. Add DNA-lipid complex to cells and mix gently to ensure even distribution.
4. Incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 3: Medium Replacement and Cell Treatment

1. Wash cells with DPBS and treat with one volume of 0.05% trypsin-EDTA. Resuspend cells in four volumes of complete medium.
2. Quantify the cells and dilute to 2×10^5 cells/ml in complete medium.
3. Plate 50µl per well into a solid, white 96-well plate (Corning Cat.# 3917).
4. Serially dilute human recombinant interleukin 6 into complete medium to give 2X stock solutions.
5. Add 50µl of the 2X dilutions of IL-6 to each well.
6. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 4: Luminescence Measurement

1. Remove plates from the 37°C, 5% CO_2 incubator and allow to cool to room temperature for approximately 15 minutes.
2. Add 100µl ONE-Glo™ detection reagent to each well and measure luminescence following the recommended protocol. (Refer to the ONE-Glo™ Luciferase Assay System Technical Manual, #TM292 for details).

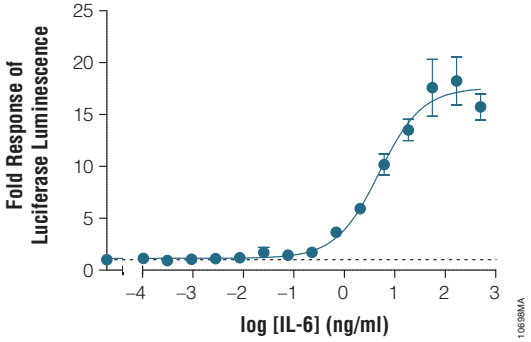


Figure 1. Representative data for pGL4.47[*luc2P*/SIE/Hygro] in HEK293 cells upon stimulation with IL-6. HEK293 cells were transiently transfected with pGL4.47[*luc2P*/SIE/Hygro] and assayed in 96-well format after 24 hours stimulation with IL-6 as indicated in the protocol. Firefly luciferase luminescence normalized to untreated cells is shown, with error bars indicating the S.E.M. for three replicates. Luminescence was detected after addition of ONE-Glo™ Reagent, using a GloMax® Multi+ instrument with a 0.5 second integration time.

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