Certificate of Analysis

pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector:

Part No.	Size
E849A	20μg

Part# 9PIE849
Revised 7/16

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

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Description: The pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector[a–c] contains five copies of an NF-κB response element (NF-κB-RE) 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. The protein encoded by *luc2P* responds more quickly than the protein encoded by the *luc2* gene upon induction. The vector backbone contains an ampicillin resistance gene to allow selection in *E. coli* and a mammalian selectable marker for hygromycin resistance.

Concentration: 1μg/μl.

GenBank® Accession Number: EU581860.

Storage Buffer: The pGL4.32[*luc2P*/NF-κB-RE/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. These fluctuations can greatly alter product stability. 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.

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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} ≥1.80, A_{260}/A_{250} ≥1.05.

Sequence: The pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors**

Signed by:

R. Wheeler, Quality Assurance

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

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

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Part# 9PIE849
Printed in USA. Revised 7/16.

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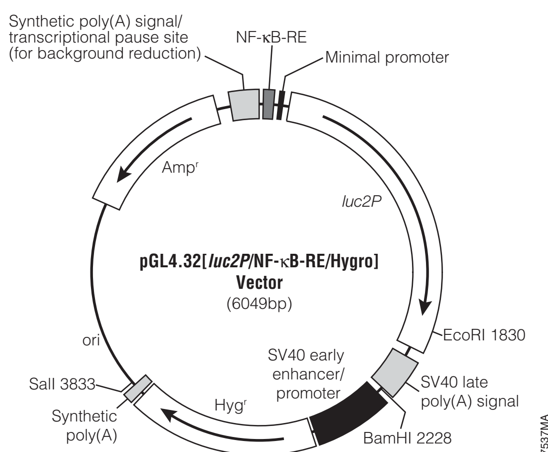
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector Features List and Map:

NF-κB response element	33–84
Minimal promoter	117–147
luc2P reporter gene	180–1955
SV40 late poly(A) signal	1995–2216
SV40 early enhancer/promoter	2264–2682
Synthetic hygromycin (Hyg^r) coding region	2707–3744
Synthetic poly(A) signal	3768–3816
Reporter Vector primer 4 (RVprimer4) binding region	3883–3902
*Col*E1-derived plasmid replication origin	4140
Synthetic β-lactamase (Amp^r) coding region	4931–5791
Synthetic poly(A) signal/transcriptional pause site	5896–6049
Reporter Vector primer 3 (RVprimer3) binding region	5998–6017



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

Sample Protocol to Determine Induction of Luciferase by TNFα in HEK 293 Cells Transfected with the pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector

Materials to be Supplied by User

- Dulbecco's PBS (DPBS)
- 0.05% (w/v) trypsin in DPBS
- DMEM
- DMEM supplemented with 10% fetal bovine serum (DMEM/FBS)
- Tumor necrosis factor-α (Sigma T0157), 10µg/ml solution in PBS containing 1mg/ml BSA
- Bright-Glo™ Luciferase Assay System (Cat.# E2610)
- HEK 293 cells

Day 1: Plate Cells

1. Grow HEK 293 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 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 96-well plate to be transfected requires 0.1µg pGL4.32[*luc2P*/NF-κB-RE/Hygro] plasmid DNA. Transfection conditions may require optimization.
2. Cover the plate and place it in a tissue culture incubator at 37°C overnight or as needed for cell recovery depending on the transfection method used. We have used 24 hours recovery time for lipid-mediated transfections.

Day 3: Induce Transfected Cells

1. Prepare 1X induction and 1X control solutions. Calculate the volume of 1X induction and 1X control solution by multiplying the number of wells needed for each solution by 100µl and prepare 110% of this amount.
   - 1X induction solution: Dilute 10µg/ml TNFα solution to 20ng/ml in DMEM/FBS. Final TNFα concentration will be 20ng/ml.
   - 1X control solution: DMEM/FBS.
2. Remove media from wells that will be treated with either 1X induction solution or 1X control solution.
3. Add 100µl of 1X induction solution to the cells to be induced and 100µl of 1X control solution to the control noninduced cells.
4. Return the plate to the tissue culture incubator and induce for 5 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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Printed in USA. Revised 7/16.