Certificate of Analysis

pGL4.44[*luc2P*/AP1 RE/Hygro] Vector:

Part No.	Size
E411A	20µg

Part# 9PIE411
Revised 9/16

Description: The pGL4.44[*luc2P*/AP1 RE/Hygro] Vector[a–c] contains six copies of an AP-1 response element (AP1 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, 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: JQ858516.

Storage Buffer: The pGL4.44[*luc2P*/AP1 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. 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 pGL4.44[*luc2P*/AP1 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

Promega Corporation

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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.

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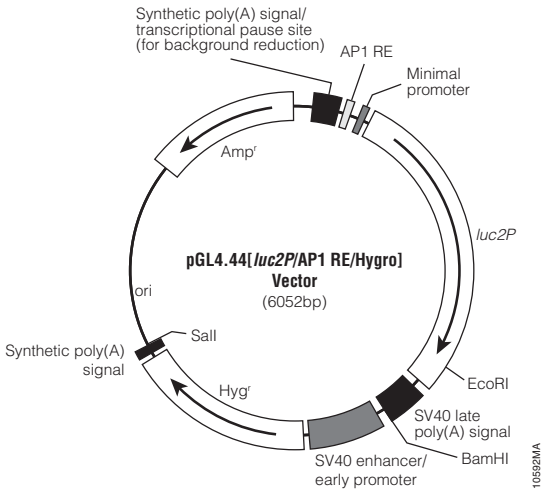
Promega

pGL4.44[*luc2P*/AP1 RE/Hygro] Vector Features List and Map:

AP1 response element	285–332
Minimal promoter	378–408
luc2P reporter gene	441–2216
SV40 late poly(A) signal	2256–2477
SV40 early enhancer/promoter	2525–2943
Synthetic hygromycin (Hyg^r) coding region	2968–4005
*Col*E1-derived plasmid replication origin	4401
Synthetic β-lactamase (Amp^r) coding region	5192–6052
Synthetic poly(A) signal sequence	4029–4077
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	4144–4163



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

Example Protocol

In this example protocol, the pGL4.44[*luc2P*/AP1 RE/Hygro] Vector is used to measure activation of the AP1 RE in HEK293 cells upon treatment with PMA. The pGL4.75 Vector (encoding *Renilla* luciferase) is used as a normalization control. 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

- Dulbecco's PBS (DPBS; Life Technologies Cat.# 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- DMEM (Life Technologies Cat.# 11995)
- complete medium (DMEM supplemented with 10% fetal bovine serum [DMEM/FBS; Life Technologies Cat.# 16000] and 1X NEAA [Life Technologies Cat.# 11140])
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- PMA (Cat.# V1171)
- Dual-Glo® Luciferase Assay System (Cat.# E2940)
- HEK293 cells
- pGL4.75[*hRluc*/CMV] Vector (Cat.# E6931)

Day 1: Reverse Transfection

Preparation of Cells

1. Grow HEK293 cells in complete medium [DMEM + 10% FBS + 1X NEAA]. Wash with DPBS and treat with one volume of 0.05% trypsin-EDTA. Resuspend cells in four volumes of complete medium.
2. Pellet the cells by centrifugation at 233 x *g* for 5 minutes in a swinging-bucket rotor. Resuspend in complete medium at a concentration of 1×10^5 cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.44[*luc2P*/AP1 RE/Hygro] and pGL4.75 [*hRluc*/CMV] *Renilla* luciferase control vector constructs in a 10:1 mass ratio, respectively, to 10ng total DNA/µl in Opti-MEM® I.
2. Add FuGENE® HD to a 3:1 lipid:DNA ratio. Mix by pipetting. Incubate at room temperature for 30 minutes.
3. Dilute lipid:DNA mixture 20-fold with 1×10^5 cells/ml cell suspension. Mix by pipetting.
4. Plate 100µl per well into a solid, white 96-well plate (Corning Cat.# 3917).
5. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Medium Replacement

1. Aspirate medium and replace with 75µl DMEM + 0.1% FBS.
2. Incubate for 17 hours in a 37°C, 5% CO_2 incubator.

Day 3: Cell Treatment and Luminescence Measurement

1. Dissolve PMA in DMSO to a final concentration of 10mM. Serially dilute this solution in DMSO to give a range of concentrated stock solutions (1,000X). Dilute each concentrated stock solution using Opti-MEM® I to give a range of dilute stock solutions (16X). Add 5µl of dilute stock solution to the existing 75µl of medium per well, covering a final concentration range of PMA from 1pM to 1µM.
2. Incubate for 6 hours in a 37°C, 5% CO_2 incubator.
3. Remove plates from the incubator and allow them to cool to room temperature for approximately 15 minutes.
4. Add Dual-Glo® Luciferase Assay System detection reagents, and measure luminescence following the recommended protocol (Refer to the Dual-Glo® Luciferase Assay System Technical Manual, #TM058 for details).

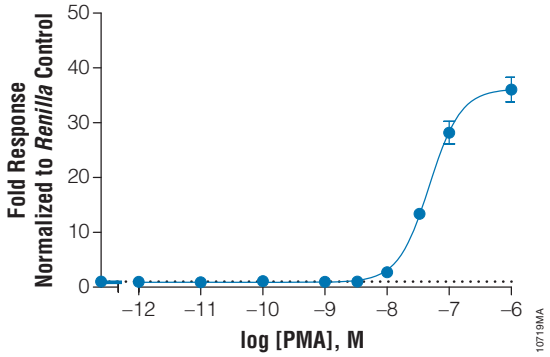


Figure 1. Representative data for pGL4.44[*luc2P*/AP1 RE/Hygro] in HEK293 cells upon stimulation with PMA. HEK293 cells were transiently transfected with pGL4.44[*luc2P*/SIE/Hygro] and assayed in a 96-well format as indicated in the protocol after six hours stimulation with PMA. Firefly luciferase luminescence normalized to the *Renilla* luciferase control is shown, with error bars indicating the S.E.M. for five replicates. Luminescence was detected after addition of Dual-Glo® reagents, using a GloMax® 96 instrument with a 0.5 second integration time.

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