

Certificate of Analysis

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

Part No. Size
E411A 20µg

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.

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

(a)BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE. If the researcher is not willing to accept the terms of this label license, and the product is unused, Promega will accept return of the unused product and provide the researcher with a full refund.

Researchers may use this product for research use only, no commercial use is allowed. "Commercial use" means any and all uses of this product and derivatives by a party for money or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. 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 provided that the coding sequence of the resulting luciferase gene has no more than four deoxynucleotides missing at the affected terminus compared to the intact luciferase gene sequence, and (2) insert and remove nucleic acid sequences in splicing research predicated on the inactivation or reconstitution of the luminescence of the encoded luciferase. No other use or transfer of this product or derivatives is authorized without the prior express written consent of Promega. In addition, 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 and its derivatives. Researchers may transfer derivatives to others for research use provided that at the time of transfer a copy of this label license is given to the recipients and recipients agree to be bound by the terms of this label license. With respect to any uses outside this label license, including any diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information. PROMEGA MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING FOR MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WITH REGARD TO THE PRODUCT. The terms of this label license shall be governed under the laws of the State of Wisconsin, USA. This label license relates to Promega patents and/or patent applications on improvements to the luciferase gene.

(b)U.S. Pat. No. 7,728,118.

(c)U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

Part# 9PIE411
Revised 9/16



AF9PIE411 0916E411



Promega

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

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 2012, 2016 Promega Corporation. All Rights Reserved.

Dual-Glo and GloMax are registered trademarks of Promega Corporation.

FuGENE is a registered trademark of Fugent, L.L.C., USA. GenBank is a registered trademark of the U.S. Department of Health and Human Services. Opti-MEM is a registered trademark of Life Technologies, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

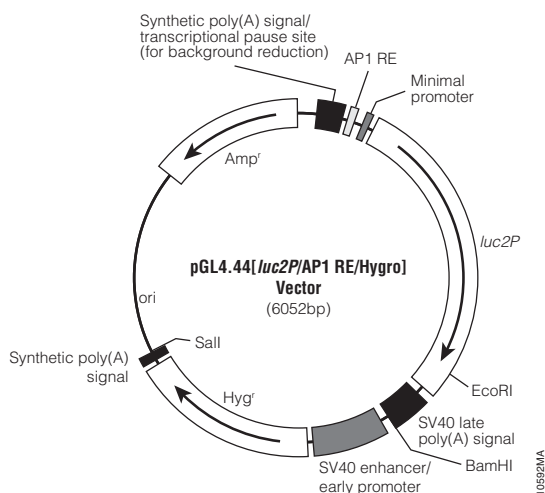
All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIE411
Printed in USA. Revised 9/16.

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

AP1 response element	285–332
Minimal promoter	378–408
<i>luc2P</i> 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
<i>ColE1</i> -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% Trypsin-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 × 10⁵ cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.44[*luc2P/AP1 RE/Hygro*] and pGL4.75 [h*Rluc*/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 × 10⁵ 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% CO₂ 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% CO₂ 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% CO₂ 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).

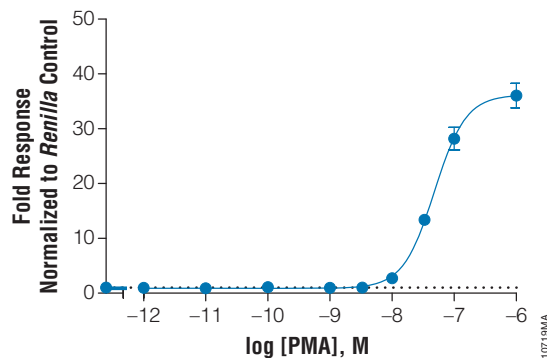


Figure 1. Representative data for pGL4.44[*luc2P/AP1 RE/Hygro*] in HEK293 cells upon stimulation with PMA. HEK293 cells were transiently transfected with pGL4.44[*luc2P/SIE/Hygro*] and assayed in a 96-well format as indicated in the protocol after six hours stimulation with PMA. Firefly luciferase luminescence 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.

Part# 9PIE411
Printed in USA. Revised 9/16.