Certificate of Analysis

pGL4.42[*luc2P*/HRE/Hygro] Vector:

Part No.	Size
E400A	20µg

Part# 9PIE400
Revised 4/18

Description: The pGL4.42[*luc2P*/HRE/Hygro] Vector[a–c] contains four copies of a hypoxia response element (HRE) 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: JQ858518.

Storage Buffer: The pGL4.42[*luc2P*/HRE/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 pGL4.42[*luc2P*/HRE/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:

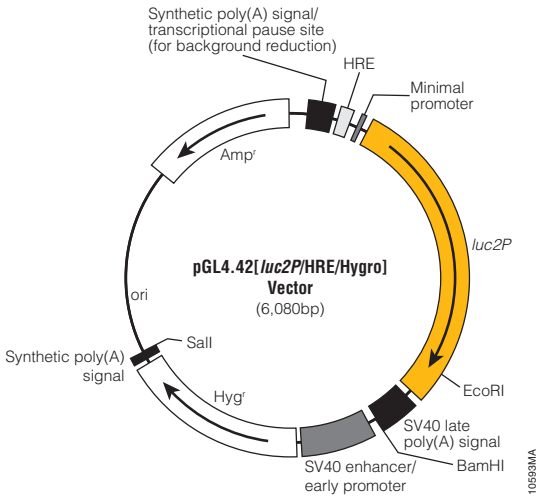
R. Wheeler, Quality Assurance

Part# 9PIE400
Printed in USA. Revised 4/18.

Promega

pGL4.42[*luc2P*/HRE/Hygro] Vector Features List and Map:

HRE response element	285–360
Minimal promoter	406–436
luc2P reporter gene	469–2244
SV40 late poly(A) signal	2284–2505
SV40 early enhancer/promoter	2553–2971
Synthetic hygromycin (Hyg^r) coding region	2996–4033
*Col*E1-derived plasmid replication origin	4429
Synthetic β-lactamase (Amp^r) coding region	5220–6080
Synthetic poly(A) signal sequence	4057–4105
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	4172–4191



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

Example Protocol

In this example protocol, the pGL4.42[*luc2P*/HRE/Hygro] Vector is used to measure activation of the HRE in HEK293 cells upon treatment with 1,10-phenanthroline. 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)
- DMSO (Sigma Cat.# D2650)
- 1,10-phenanthroline (Sigma Cat.# 131377))
- 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 pGL4.42[*luc2P*/HRE/Hygro] and pGL4.75 [*hRluc*/CMV] *Renilla* luciferase 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: Cell Treatment and Luminescence Measurement

1. Dissolve 1,10-phenanthroline to a final concentration of 50mM in DMSO. Serially dilute this solution using DMSO to give a range of concentrated stock solutions (500X). Dilute each concentrated stock solution using Opti-MEM® I to give a range of dilute stock solutions (10X). Add 10µl of the 10X stocks to each well.
2. Incubate for 5 hours in a 37°C, 5% CO_2 incubator.
3. Remove plates from the 37°C, 5% CO_2 incubator. Allow plates 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).

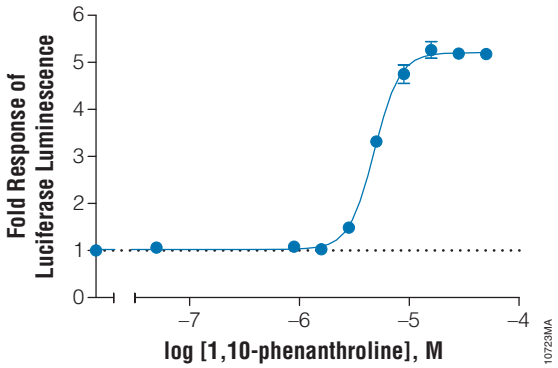


Figure 1. Representative data for pGL4.42[*luc2P*/HRE/Hygro] in HEK293 cells upon stimulation with 1,10-phenanthroline. HEK293 cells were transiently transfected with pGL4.42[*luc2P*/HRE/Hygro] and pGL4.75 and assayed in 96-well format after five hours stimulation with 1,10-phenanthroline as indicated in the protocol. Firefly luciferase luminescence is shown, normalized to untreated cells, with error bars indicating the S.E.M. for six replicates. Luminescence was detected after addition of Dual-Glo® reagent, using a GloMax® 96 instrument with a 0.5 second integration time.

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Printed in USA. Revised 4/18.