Certificate of Analysis

pGL4.37[*luc2P*/ARE/Hygro] Vector:

Part No.	Size
E364A	20µg

Part# 9PIE364
Revised 4/18

Description: The pGL4.37[*luc2P*/ARE/Hygro] Vector[a–c] contains four copies of an antioxidant response element (ARE) 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: JQ858521.

Storage Buffer: The pGL4.37[*luc2P*/ARE/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 pGL4.37[*luc2P*/ARE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors/**

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

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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.

Signed by:

R. Wheeler, Quality Assurance

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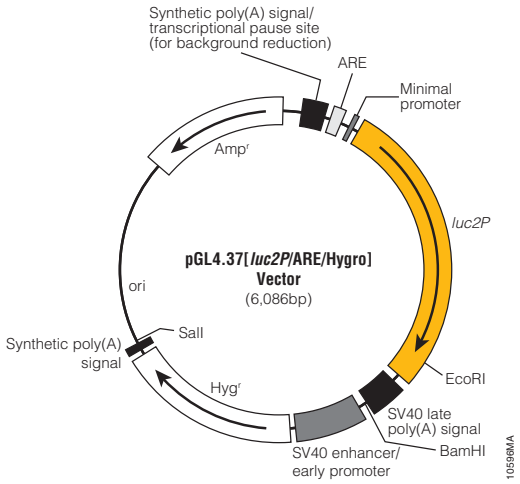
Promega

pGL4.37[*luc2P*/ARE/Hygro] Vector Features List and Map:

ARE response element	285–366
Minimal promoter	412–442
luc2P reporter gene	475–2250
SV40 late poly(A) signal	2290–2511
SV40 early enhancer/promoter	2559–2977
Synthetic hygromycin (Hyg^r) coding region	3002–4039
*Col*E1-derived plasmid replication origin	4435
Synthetic β-lactamase (Amp^r) coding region	5226–6086
Synthetic poly(A) signal sequence	4063–4111
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	4178–4197



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

Example Protocol

In this example protocol, the pGL4.37[*luc2P*/ARE/Hygro] Vector is used to measure activation of the ARE in HEK293 cells upon treatment with tert-Butylhydroquinone or D,L-Sulforaphane. 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 [Life Technologies Cat.# 11995] and 1X NEAA [Life Technologies Cat.# 11140])
- Charcoal-stripped FBS (Life Technologies Cat.# 126776-011)
- DMSO (Sigma Cat.# D2650)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- tert-Butylhydroquinone (Sigma Cat.# 112941) or D,L-Sulforaphane (Sigma Cat.# S4441)
- Dual-Glo® Luciferase Assay System (Cat.# E2940)
- HEK293 cells
- pGL4.75[*hRluc*/CMV] Vector (Cat.# E6931)

Day 1: Plate 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. Quantify the cells and dilute to 1.5×10^5 cells/ml in complete medium.
3. Plate 100µl per well to a solid, white 96-well plate (Corning Cat.# 3917).
4. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Transfection

1. Dilute pGL4.37[*luc2P*/ARE/Hygro] and pGL4.75 [*hRluc*/CMV] *Renilla* luciferase vector constructs in a 10:1 mass ratio, respectively, to 12.5ng 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 20 minutes.
3. Add 8µl transfection complex per well (100ng DNA/well) and incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 3: Medium Replacement and Cell Treatment

1. Remove medium from cells and replace with 72µl of DMEM + 0.5% charcoal-stripped FBS per well.
2. Incubate for 6 hours in a 37°C, 5% CO_2 incubator.
3. Resuspend tert-Butylhydroquinone (tBHQ) to 500mM in ethanol. Serially dilute into ethanol to give 1,000X stocks. Resuspend D,L-Sulforaphane to 200mM in DMSO. Serially dilute into DMSO to give 1,000X stocks. Dilute the 1,000X stocks into Opti-MEM® I to give 10X stocks.
4. Add 8 µl of the 10X stocks of tBHQ or D,L-Sulforaphane to each well and incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 4: Luciferase 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 80µl of the 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).

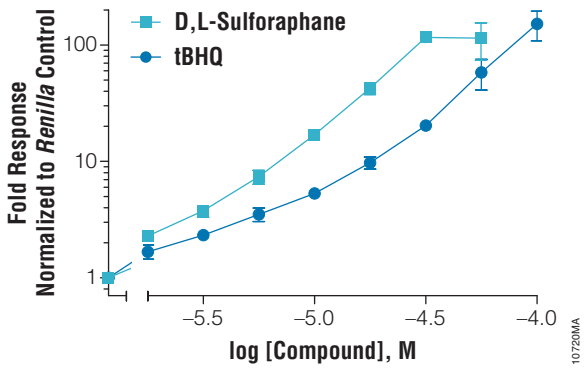


Figure 1. Representative data for pGL4.37[*luc2P*/ARE/Hygro] in HEK293 cells upon stimulation with tBHQ or D,L-Sulforaphane. HEK293 cells were transiently transfected with pGL4.37[*luc2P*/ARE/Hygro] and pGL4.75 and assayed in 96-well format after 18 hours stimulation with tBHQ or D,L-Sulforaphane as described. Firefly luciferase luminescence normalized to the *Renilla* luciferase control is shown, with error bars indicating the S.E.M. for six 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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