Certificate of Analysis

pGL4.39[*luc2P*/ATF6 RE/Hygro] Vector:

Part No.	Size
E366A	20µg

Part# 9PIE366
Revised 4/18

Description: The pGL4.39[*luc2P*/ATF6 RE/Hygro] Vector[a–c] contains five copies of an ATF6 response element (ATF6 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: JQ858519.

Storage Buffer: The pGL4.39[*luc2P*/ATF6 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.

AF9PIE366 0418E366

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 pGL4.39[*luc2P*/ATF6 RE/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

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE. 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, and (2) insert and remove nucleic acid sequences in splicing research. No other use or transfer of this product or derivatives is authorized. 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. For any uses outside this label license, contact Promega for supply and licensing information. This product is for research use only; no commercial use is allowed. For a full copy of this label license, including the definition of "commercial use," go to: www.promega.com/LULL

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

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

Signed by:

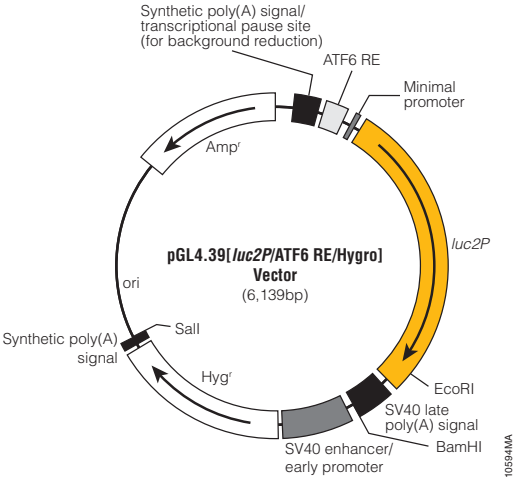
R. Wheeler, Quality Assurance

Part# 9PIE366
Printed in USA. Revised 4/18.

Promega

pGL4.39[*luc2P*/ATF6 RE/Hygro] Vector Features List and Map:

ATF6 response element	285–419
Minimal promoter	465–495
luc2P reporter gene	528–2303
SV40 late poly(A) signal	2343–2564
SV40 early enhancer/promoter	2612–3030
Synthetic hygromycin (Hyg^r) coding region	3055–4092
*Col*E1-derived plasmid replication origin	4488
Synthetic β-lactamase (Amp^r) coding region	5279–6139
Synthetic poly(A) signal sequence	4116–4164
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	4231–4250



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

Example Protocol

In this example protocol, the pGL4.39[*luc2P*/ATF6 RE/Hygro] Vector is used to measure activation of the ATF6 RE in HeLa cells upon treatment with tunicamycin.. 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

- 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])
- Dulbecco's PBS (DPBS; Life Technologies Cat. # 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- Charcoal-stripped FBS (Life Technologies Cat.# 126776-011)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- Tunicamycin (Calbiochem Cat.# 654380)
- DMSO (Sigma Cat.# D2650)
- Dual-Glo® Luciferase Assay System (Cat.# E2940)
- HeLa cells
- pGL4.75[*hRluc*/CMV] Vector (Cat.# E6931)

Day 1: Reverse Transfection

Preparation of Cells

1. Grow HeLa 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 200 × *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 pGL4.39[*luc2P*/ATF6 RE/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 and mix by inversion.
4. Plate 100µl per well into a solid, white 96-well plate (Corning Cat.# 3917).
5. Incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 2: Medium Replacement and Cell Treatment

1. Resuspend tunicamycin to 10mM in DMSO. Serially dilute into DMSO to give 1,000X stocks. Dilute 100-fold into DMEM to give 10X stocks.
2. Remove existing medium from cells and replace with 72µl of DMEM + 0.5% charcoal-stripped FBS per well.
3. Add 8µl of the 10X dilutions of tunicamycin and incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 3: Luminescence Measurement

1. Remove plates from the 37°C, 5% CO_2 incubator and allow to cool to room temperature for approximately 15 minutes.
4. 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).

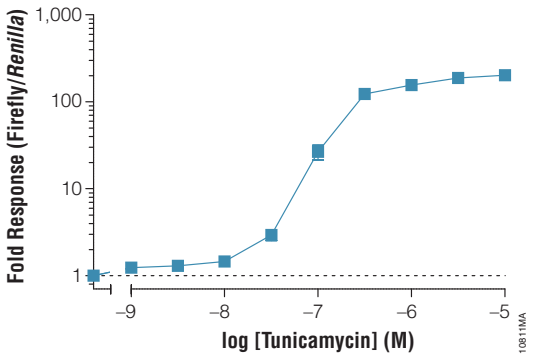


Figure 1. Representative data for pGL4.39[*luc2P*/ATF6 RE/Hygro] in HeLa cells upon stimulation with tunicamycin. HeLa cells were transiently transfected with pGL4.39[*luc2P*/ATF6 RE/Hygro] and pGL4.75 and assayed in 96-well format after 18 hours stimulation with tunicamycin as indicated in the protocol. 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.

Part# 9PIE366
Printed in USA. Revised 4/18.