Certificate of Analysis

pGL4.38[*luc2P*/p53 RE/Hygro] Vector:

Part No.	Size
E365A	20µg

Part# 9PIE365
Revised 4/18

Description: The pGL4.38[*luc2P*/p53 RE/Hygro] Vector[a–c] contains two copies of a p53 response element (p53 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: JQ858522.

Storage Buffer: The pGL4.38[*luc2P*/p53 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 pGL4.38[*luc2P*/p53 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

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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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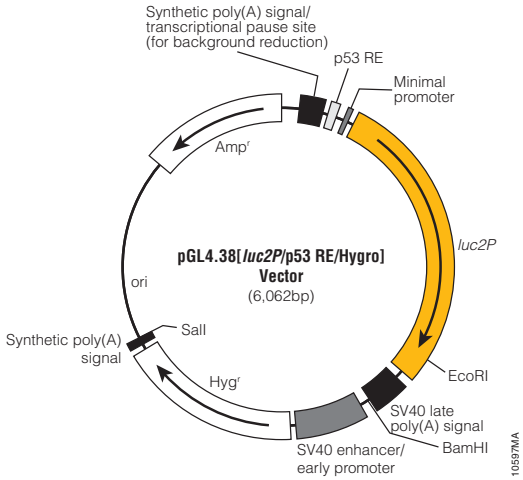
Promega

pGL4.38[*luc2P*/p53 RE/Hygro] Vector Features List and Map:

p53 response element	285–342
Minimal promoter	388–418
luc2P reporter gene	451–2226
SV40 late poly(A) signal	2266–2487
SV40 early enhancer/promoter	2535–2953
Synthetic hygromycin (Hyg^r) coding region	2978–4015
*Col*E1-derived plasmid replication origin	4411
Synthetic β-lactamase (Amp^r) coding region	5202–6062
Synthetic poly(A) signal sequence	4039–4087
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	4154–4173



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

Example Protocol

In this example protocol, the pGL4.38[*luc2P*/p53 RE/Hygro] Vector is used to measure activation of the p53 RE in U2OS cells upon treatment with doxorubicin, etoposide, nutlin-3, or mitomycin c. 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

- Complete medium [McCoy's 5A (Life Technologies Cat.# 16600) + 10% FBS (Life Technologies Cat.# 16000) + 1X NEAA (Life Technologies Cat.# 11140) + 1X sodium pyruvate (Life Technologies Cat.# 11360)]
- 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)
- Doxorubicin (Sigma Cat.# D1515)
- Etoposide (Calbiochem Cat.# 341205)
- Mitomycin c (Sigma Cat.# 705436)
- Nutlin-3 (Sigma Cat.# N6287)
- DMSO (Sigma Cat.# D2650)
- Dual-Glo® Luciferase Assay System (Cat.# E2940)
- U2OS cells
- pGL4.75[*hRluc*/CMV] Vector (Cat.# E6931)

Day 1: Plate Cells

1. Grow U2OS cells in complete medium (McCoy's 5A + 10% FBS + 1X NEAA + 1X sodium pyruvate). Wash with DPBS and treat with one volume of 0.05% trypsin-EDTA. Resuspend the cells in four volumes of complete medium.
2. Quantify the cells and dilute to 1×10^5 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 pGL4.38[*luc2P*/p53 RE/Hygro] and pGL4.75 [*hRluc*/CMV] *Renilla* luciferase control 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 24 hours in a 37°C, 5% CO_2 incubator.

Day 3: Medium Replacement and Cell Treatment

1. Resuspend doxorubicin to 50mM in water. Resuspend etoposide to 50mM in DMSO. Resuspend mitomycin c to 1mM in water. Resuspend nutlin-3 to 10mM in DMSO. Make serial dilutions in either water or DMSO and then dilute into Opti-MEM® I to make 10X stocks.
2. Remove existing medium from cells and replace with 72µl of McCoy's 5A + 0.5% charcoal-stripped FBS per well.
3. Add 8µl of the 10X compound dilutions and incubate for 18 or 40 hours in a 37°C, 5% CO_2 incubator.

Day 4: Luminescence 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).

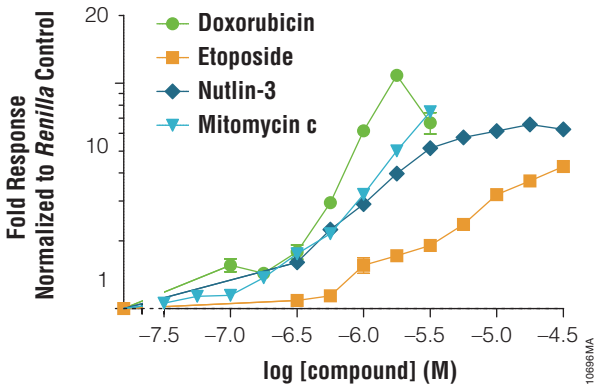


Figure 1. Representative data for pGL4.38[*luc2P*/p53 RE/Hygro] in U2OS cells upon stimulation with doxorubicin, etoposide, nutlin-3 and mitomycin c. U2OS cells were transiently transfected with the pGL4.38[*luc2P*/p53 RE/Hygro] Vector and pGL4.75, and assayed in 96-well format after 18 hours stimulation with doxorubicin, nutlin-3, and etoposide, or after 40 hours with mitomycin c 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.

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