Certificate of Analysis

pGL4.45[*luc2P*/ISRE/Hygro] Vector:

Part No.	Size
E414A	20µg

Part# 9PIE414
Revised 4/18

Description: The pGL4.45[*luc2P*/ISRE/Hygro] Vector[a–c] Vector contains five copies of an interferon-stimulated response element (ISRE) 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: JQ858514.

Storage Buffer: The pGL4.45[*luc2P*/ISRE/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 Notes:

This plasmid may show instability when propagated in certain strains of *E. coli*, losing one or more of the ISRE repeats upstream of the minimal promoter. We recommend propagating this strain in STBL2 cells (Life Technologies Cat. # 10268-019) at 30°C.

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 pGL4.45[*luc2P*/ISRE/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

Promega Corporation

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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.

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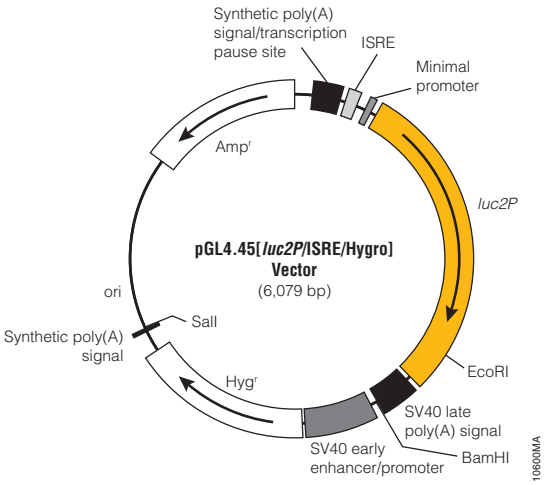
pGL4.45[*luc2P*/ISRE/Hygro] Vector Features List and Map:

ISRE response element	285–359
Minimal promoter	405–435
luc2P reporter gene	468–2243
SV40 late poly(A) signal	2283–2504
SV40 early enhancer/promoter	2552–2970
Synthetic hygromycin (Hyg^r) coding region	2995–4032
*Col*E1-derived plasmid replication origin	4428
Synthetic β-lactamase (Amp^r) coding region	5219–6079
Synthetic poly(A) signal sequence	4056–4104
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	4171–4190



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

Example Protocol

In this example protocol, the pGL4.45[*luc2P*/ISRE/Hygro] Vector is used to measure activation of the ISRE in U2OS cells upon treatment with interferon-alpha. 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 [HyClone Cat.# SH30070.03])
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- Dulbecco's PBS (DPBS; Life Technologies Cat.# 14190)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- Interferon-alpha (IFN-alpha; EMD Cat.# 407294))
- ONE-Glo® Luciferase Assay System (Cat.# E6120)
- U2OS cells

Day 1: Reverse Transfection

Preparation of Cells

1. Grow U2OS cells in complete medium (McCoy's 5A + 10% FBS). Wash with DPBS and treat with one volume of 0.05% trypsin-EDTA. Resuspend the cells in four volumes of complete medium.
2. Quantify cells and dilute in complete medium to 2×10^5 cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.45[*luc2P*/ISRE/Hygro] DNA 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 2×10^5 cells/ml cell suspension. 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: Cell Treatment

1. Serially dilute IFN-alpha into complete medium to give 10X stock solutions.
2. Add 10µl of the 10X dilutions of IFN-alpha to each well.
3. Incubate for 16 hours in a 37°C, 5% CO_2 incubator.

Day 3: Luminescence Measurement

1. Remove plates from the 37°C, 5% CO_2 incubator. Allow plates to cool to room temperature for approximately 15 minutes.
2. Add 100µl ONE-Glo® Luciferase Assay System detection reagent to each well and measure luminescence following the recommended protocol (Refer to the ONE-Glo™ Luciferase Assay System Technical Manual, #TM292 for details).

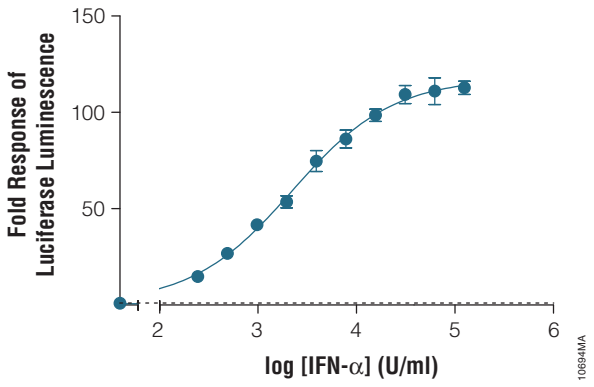


Figure 1. Representative data for pGL4.45[*luc2P*/ISRE/Hygro] in U2OS cells upon stimulation with IFN-alpha. U2OS cells were transiently transfected with pGL4.45[*luc2P*/ISRE/Hygro] and assayed in 96-well format after 16 hours stimulation with IFN-alpha as indicated in the protocol. Firefly luciferase luminescence normalized to untreated cells is shown, with error bars indicating the S.E.M. for three replicates. Luminescence was detected after addition of ONE-Glo® reagent, using a GloMax®-Multi+ instrument with a 0.5 second integration time.

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