Certificate of Analysis

pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector:

Part No.	Size
E465A	20µg

Part# 9PIE465
Revised 4/18

AF9PIE465 0418E465

Description: The pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector[a–c] contains five copies of a STAT5 response element (STAT5 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: JX206457.

Storage Buffer: The pGL4.52[*luc2P*/STAT5 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.

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 pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors/**

Promega

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Signed by:

R. Wheeler, Quality Assurance

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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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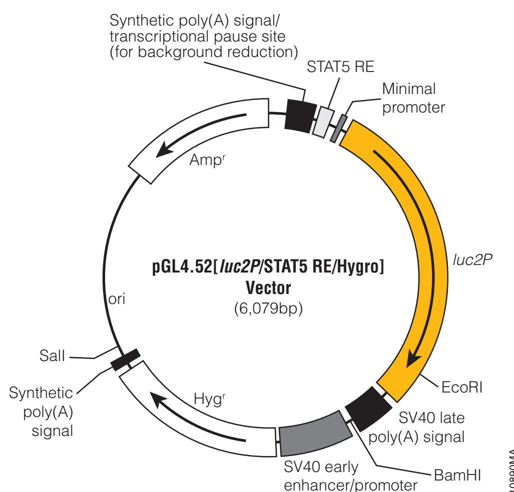
Part# 9PIE465
Printed in USA. Revised 4/18.

pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector Features List and Map:

STAT5 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 pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector is used to measure activation of the STAT5 RE in Ba/F3 cells upon treatment with mIL-3. 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)
- Complete medium (RPMI Medium [Life Technologies Cat.# 22400] + 10% heat-inactivated FBS [Life Technologies Cat.# 10082-139] + 1 ng/ml mIL-3 [R&D Systems Cat.# 403-ML])
- Opti-MEM® I (Life Technologies Cat.# 11058)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- mIL-3 (R&D Systems Cat.# 403-ML)
- BSA (Proliant Cat.# 68700)
- ONE-Glo® Luciferase Assay System (Cat.# E6120)
- Ba/F3 cells

Day 1: Reverse Transfection

Preparation of Cells

1. Grow Ba/F3 cells in suspension in complete medium [RPMI Medium + 10% heat-inactivated FBS + 1ng/ml mIL-3].
2. Quantify cells and dilute them in complete medium to 1×10^5 cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector to 10ng total DNA/µl in Opti-MEM® I.
2. Add FuGENE® HD to a 6:1 lipid:DNA ratio and mix gently. Incubate at room temperature for 15 minutes.
3. Dilute lipid:DNA mixture 20-fold with 1×10^5 cells/ml cell suspension and mix by inversion.
4. Place cells in a flask and incubate for 18–24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Plating, Cell Treatment and Luminescence Measurement

Plating Cells

1. Pellet the cells by centrifugation at 200 × *g* for 5 minutes in a swinging-bucket rotor. Wash once with DPBS and spin again.
2. Resuspend cells in Opti-MEM® I to 1×10^6 cells/ml.
3. Plate 100µl per well to a solid, white 96-well plate (Corning Cat.# 3917).
4. Incubate for 4 hours in a 37°C, 5% CO_2 incubator.

Cell Treatment

1. Resuspend mIL-3 to 0.1 mg/mL in DPBS + 0.1% BSA. Make serial dilutions into Opti-MEM® I to make 10X stocks.
2. Add 10µl of the 10X stocks of mIL-3 to each well and incubate for 4 hours in a 37°C, 5% CO_2 incubator.

Luminescence Measurement

3. Remove plates from the 37°C, 5% CO_2 incubator and allow them to cool to room temperature for approximately 15 minutes.
4. Add 100µl of 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).

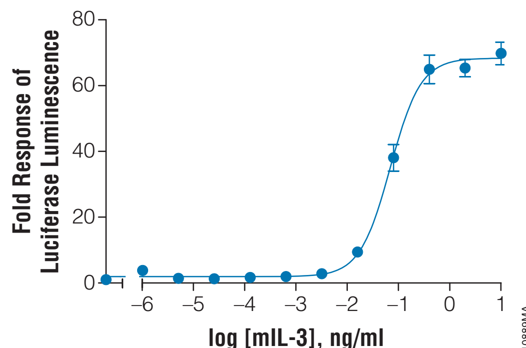


Figure 1. Representative data for pGL4.52[luc2P/STAT5 RE/Hygro] in Ba/F3 cells upon stimulation with mIL-3. Ba/F3 cells were transiently transfected with pGL4.52[*luc2P*/STAT5 RE/Hygro] Vector and assayed in 96-well format after 4 hours stimulation with mIL-3 as indicated in the protocol. Firefly luciferase luminescence normalized to untreated cells is shown, with error bars indicating the S.E.M. for four replicates. Luminescence was detected after addition of ONE-Glo® reagent, using a GloMax® Multi+ instrument with a 0.5-second integration time.

Part# 9PIE465
Printed in USA. Revised 4/18.