Certificate of Analysis

pGL4.36[*luc2P*/MMTV/Hygro] Vector

Part No.	Size
E136A	20µg

Part# 9PIE136

Printed 3/09

Instructions for use of this product can be found in the pGL4 Luciferase Reporter Vectors Technical Manual #TM259, available online at: **www.promega.com/tbs**

Description: The pGL4.36[*luc2P*/MMTV/Hygro] Vector[a–e] contains MMTV LTR (Murine Mammary Tumor Virus Long Terminal Repeat) that drives the transcription of the luciferase reporter gene *luc2P* in response to activation of several nuclear receptors such as Glucocorticoid Receptor and Androgen Receptor. *luc2P* is a synthetically derived luciferase sequence with humanized codon optimization. The *luc2P* gene also contains hPEST, a protein destabilization sequence. The protein encoded by *luc2P* responds more quickly to induction than the protein encoded by the *luc2* gene. The vector backbone contains an ampicillin resistance gene to allow for selection in *E. coli* and the mammalian-selectable marker for hygromycin resistance.

Concentration: 1µg/µl.

GenBank® Accession Number: FJ773214.

Storage Buffer: The pGL4.36[*luc2P*/MMTV/Hygro] Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

Storage Conditions: See the Product Information Label for storage temperature recommendations and expiration date.

Usage Note: Mix well prior to use.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acid Assay: RNA, single-stranded DNA and chromosomal DNA are not evident in a specified sample of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of this 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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: www.promega.com/vectors

Restriction Digestion: The functional purity of this vector DNA is verified by complete digestion with several restriction enzymes at 37°C for 1 hour. Samples are examined by agarose gel electrophoresis, and cut and uncut vector DNA are compared with marker DNA.

Signed by: J. Stevens

J. Stevens, Quality Assurance

[a]READ THIS FIRST BEFORE OPENING PRODUCT
The sale of this product and its use by the purchaser are subject to the terms of a limited use label license, the full text of which is shipped with this product and also available at: **www.promega.com\LULL**. That text must be read by the purchaser prior to opening this product to determine whether the purchaser agrees that all use of the product shall be in accordance with the license terms. If the purchaser is not willing to accept the terms of the limited use label license, Promega is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, if the product is opened for any reason, then the purchaser agrees to be bound by the terms of the limited use label license.

[b]Australian Pat. No. 2001 285278 and other patents pending.

[c]U.S. Pat. No. 5,670,356.

[d]Australian Pat. No. 2003272419 and other patents pending.

[e]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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Features List and Map for the pGL4.36[*luc2P*/MMTV/Hygro] Vector

MMTV LTR	14–1328
luc2P reporter gene	1377–3152
SV40 late poly(A) signal	3192–3413
SV40 early poly(A) enhancer/promoter	3461–3879
synthetic hygromycin (Hyg^r) coding region	3904–4941
synthetic poly(A) region	4965–5013
reporter vector primer 4 (RVprimer4) binding region	5080–5099
ColE1-derived plasmid replication origin	5337
synthetic β-lactamase (Amp^r) coding region	6128–6988
synthetic poly(A) signal/transcription pause site	7093–7246
reporter vector primer 3 (RVprimer3) binding region	7195–7214

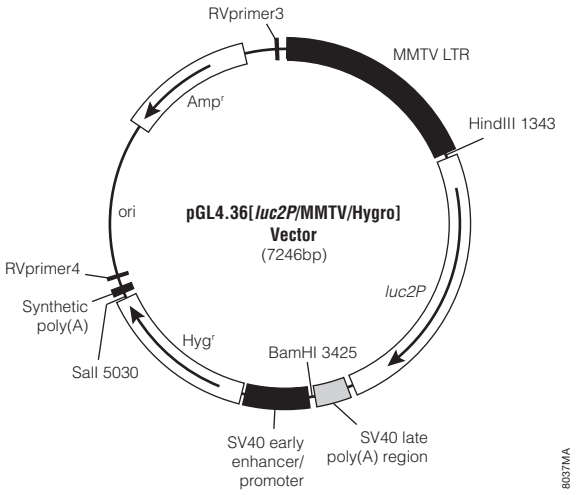

Figure 1. pGL4.36[*luc2P*/MMTV/Hygro] Vector map.

Sequence information, vector maps and restriction enzyme tables for the pGL4 Vectors are available online at: **www.promega.com/vectors**

Additional information is available in Technical Manual #TM259, available online at: **www.promega.com/tbs** or by request.

Sample Protocol to Determine Induction of Luciferase by Dexamethasone in HeLa Cells Transfected with the pGL4.36[*luc2P*/MMTV/Hygro] Vector

Materials to Be Supplied by the User

- 1X PBS
- 0.05% (w/v) trypsin
- DMEM with 10% fetal bovine serum (growth medium)
- DMEM without phenol red
- DMEM without phenol red supplemented with 5% charcoal/dextran-treated fetal bovine serum (assay medium)
- dexamethasone (Sigma Cat.# D4902), 10mM solution in ethanol
- Bright-Glo™ Luciferase Assay System (Cat.# E2610)
- transfection reagent
- HeLa cells

Day 1: Plate Cells

Seed HeLa cells at 10,000 cells/ well in a solid white 96-well tissue culture-treated plate using phenol red-free DMEM containing 5% charcoal/dextran-treated FBS (80µl/well).

Day 2: Transfect Cells

1. Transfect the cells using a high-efficiency transfection reagent. Each well of 96-well plate to be transfected requires 0.1µg of pGL4.36[*luc2P*/MMTV/Hygro] plasmid DNA. Transfection conditions may require optimization. We have routinely added approximately 10µl/well of a transfection master mix.
2. Cover the plate and place it in a tissue culture incubator at 37°C overnight or as needed for cell recovery depending on the transfection method used. We use 24 hours recovery time for lipid-mediated transfections.

Day 3: Induce Transfected Cells

1. Prepare 10X induction and 10X control solution. Calculate the volume of 10X induction and 10X control solution by multiplying the number of wells needed for each solution by 10µl and prepare 110% of this amount.
   - 10X induction solution: Dilute 10mM dexamethasone solution in DMEM without phenol red to 100µM (1:100 dilution). Final dexamethasone concentration will be 10µM.
   - Control solution: Add the same volume of ethanol only used in 10X induction solution to DMEM without phenol red.
2. Add 10µl of 10X induction solution to wells to be induced or control solution to control noninduced wells.
3. Return the plate to the tissue culture incubator and induce for overnight to 24 hours.

Day 4: Harvest and Analyze Luciferase Activity

1. Analyze luciferase activity using an appropriate luciferase detection assay. We have observed comparable results for fold induction of the vector using a variety of luciferase reagents, including: Bright-Glo™ Luciferase Assay System (Cat.# E2610, Technical Manual #TM052); ONE-Glo™ Luciferase Assay System (Cat.# E6110, Technical Manual #TM292); Dual-Luciferase® Reporter Assay System (Cat.# E1910, Technical Manual #TM040); and Dual-Glo® Luciferase Assay System (Cat.# E2920, Technical Manual #TM058).
2. Calculate the fold induction as follows:

$$\text{fold induction} = \frac{\text{average relative light units of induced cells}}{\text{average relative light units of control cells}}$$

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