

Certificate of Analysis

pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector

Part No. Size
E137A 20µg

Description: The pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector^(a,b,c,d,e) contains 9 repeats of GAL4 UAS (Upstream Activator Sequence). This sequence drives the transcription of the luciferase reporter gene *luc2P* in response to binding of a fusion protein containing the Gal4 DNA Binding Domain, e.g., the Estrogen Receptor (alpha) Ligand Binding Domain in the pBIND-ER α Vector (Cat.# E1390) when activated by a ligand.

Concentration: 1µg/µl.

GenBank® Accession Number: GQ229577.

Storage Buffer: 10mM Tris-HCl, 1mM EDTA (pH 7.4 at 25°C).

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

Usage Note: This product has been purified using a method that may allow transfection in some cell lines. For optimal performance, we recommend transforming this product into bacteria and purifying plasmid DNA with a method suitable for the intended use.

Part# 9PIE137

6/09



Quality Control Assays

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 selected 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.

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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Signed by:

J. Stevens, Quality Assurance

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Researchers shall have no right to modify or otherwise create variations of the nucleotide sequence of the luciferase gene except that Researchers may: (1) clone heterologous DNA sequences at either or both ends of said luciferase gene so as to create fused gene sequences provided that the coding sequence of the resulting luciferase gene has no more than four deoxynucleotides missing at the affected terminus when compared to the intact luciferase gene sequence, and (2) insert and remove nucleic acid sequences in furtherance of splicing research predicated on the inactivation or reconstitution of the luminescent activity of the encoded luciferase. In addition, Researchers must do one of the following: (1) use luminescent assay reagents purchased from Promega Corporation for all determinations of luminescence activity resulting from the research use of this product and its derivatives; or (2) contact Promega to obtain a license for the use of the product and its derivatives. No other use or transfer of this product or its derivatives is authorized without the express written consent of Promega including, without limitation, Commercial Use. Commercial Use means any and all uses of this product and derivatives by a party for monetary or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. With respect to such Commercial Use, or any diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information. If the purchaser is not willing to accept the conditions of this limited use statement, Promega is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, in the event the product is opened, then the purchaser agrees to be bound by the conditions of this limited use statement. The above license relates to Promega patents and/or patent applications on improvements to the luciferase gene.

(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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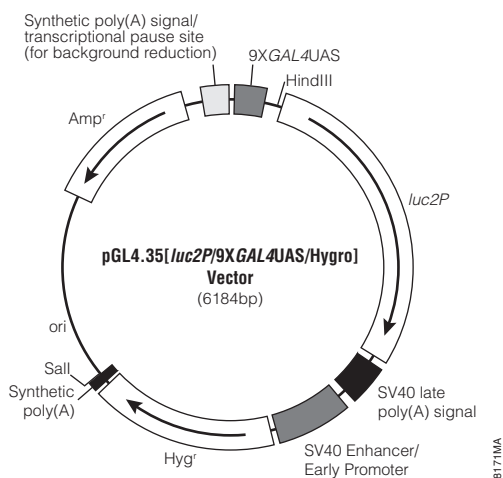
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pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector Features List and Map:

9XGAL4 UAS	31–215
Adenovirus late promoter	223–262
<i>luc2P</i> reporter gene	315–2090
SV40 late poly(A) region	2130–2351
SV40 early enhancer/promoter	2399–2817
Synthetic hygromycin (Hyg ^r) coding region	2842–3879
Synthetic poly(A) region	3903–3951
<i>Co</i> /E 1-derived plasmid origin of replication (ori)	4275–4311
Synthetic β-lactamase (Amp ^r) coding region	5066–5926
Synthetic poly(A)/transcriptional pause region	6031–6184



pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector Map.

Sequence information is available online at: www.promega.com/vectors

Sample Protocol to Determine Induction of Luciferase by Dexamethasone or β-Estradiol in HEK293 Cells Transfected with the pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector

Materials to be Supplied by User

- 1X PBS
- 0.05% (w/v) trypsin without phenol red
- 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 D4902), 10mM solution in ethanol
- β-estradiol, E2 (Sigma E2758), 100μM solution in ethanol
- luciferase assay system (e.g., Bright-Glo™ Luciferase Assay System, Cat.# E2610; see Section 6.B, Related Products, in the *GloResponse™* 9XGAL4UAS-luc2P HEK293 Cell Line Technical Bulletin, #TB552 for additional assays.)
- high-efficiency transfection reagent
- HEK293 cells
- plasmid containing a Gal4 DNA Binding Domain fusion such as pBIND-ERα Vector (Cat.# E1390) or pBIND-GR Vector (Cat.# E1581)

Day 1: Plate Cells

Seed HEK293 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).

Note: Use phenol red-free trypsin to dissociate cells, or pellet and wash cells twice with PBS to remove the phenol red.

Day 2: Transfect Cells

1. Transfect the cells using a high-efficiency transfection reagent. Each well of the 96-well plate to be transfected requires 50ng each of pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector and a fusion gene containing the Gal4 DNA Binding Domain, such as pBIND-ERα Vector (Cat.# E1390) or pBIND-GR Vector (Cat.# E1581). Use a 1:1 ratio of the two vectors. 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 have used 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. Use DMEM without phenol red and without FBS for all induction and control solutions.
 - **10X induction solution:** For the pBIND-GR Vector, dilute 10mM dexamethasone solution in phenol red-free DMEM to 100μM (1:100 dilution). Final dexamethasone concentration in the wells will be 10μM. For the pBIND-ERα Vector, dilute 100μM E2 solution in phenol red-free DMEM to 100nM (1:1,000 dilution). Final E2 concentration in the wells will be 10nM.
 - **10X control solution:** 1% ethanol in phenol red-free DMEM for the pBIND-GR Vector; 0.1% ethanol in phenol red-free DMEM for the pBIND-ERα Vector.
2. Add 10μl of 10X induction solution to wells to be induced or control solution to non-induced wells.
3. Return the plate to the tissue culture incubator and induce for overnight to 24 hours.

Day 4: Read Luminescence

1. Analyze luciferase activity using an appropriate luciferase detection assay.
2. Using the luminescence information, calculate the fold induction as follows:

$$\text{Fold Induction} = \frac{\text{Average relative light units of induced cells}}{\text{Average relative light units of control cells}}$$