Certificate of Analysis

pGL4.35[*luc2P*/9X*GAL4*UAS/Hygro] Vector

Part No.	Size
E137A	20µg

Part# 9PIE137
Revised 10/16

Description: The pGL4.35[*luc2P*/9X*GAL4*UAS/Hygro] Vector[a–c] contains 9 repeats of GAL4 UAS (Upstream Activator Sequence). This sequence drives 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-ERα 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 was 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.

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

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[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE.

Researchers shall have no right to modify or otherwise create variations of the nucleotide sequence of the luciferase gene except that researchers may (1) create fused gene sequences, and (2) insert and remove nucleic acid sequences in splicing research. No other use or transfer of this product or derivatives is authorized. Researchers must either (1) use luminescent assay reagents purchased from Promega for all determinations of luminescence activity of this product and its derivatives; or (2) contact Promega to obtain a license for use of the product. For any uses outside this label license, contact Promega for supply and licensing information. This product is for research use only; no commercial use is allowed. For a full copy of this label license, including the definition of "commercial use," go to: **www.promega.com/LULL**

[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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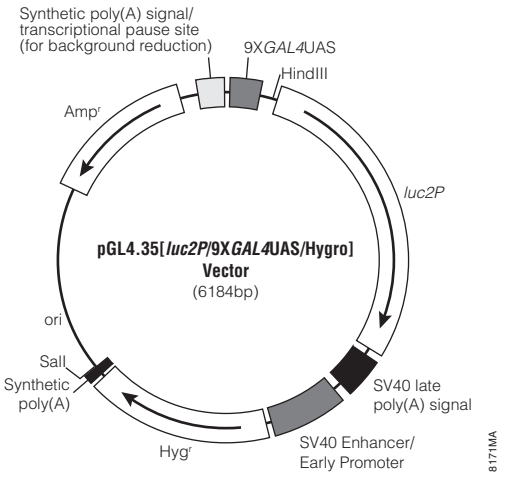
Promega

pGL4.35[*luc2P*/9X*GAL4*UAS/Hygro] Vector Features List and Map:

9X*GAL4* UAS	31–215
Adenovirus late promoter	223–262
luc2P 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
*Co*lE1-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*/9X*GAL4*UAS/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*/9X*GAL4*UAS/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 the Related Products in the *GloResponse™ 9X*GAL4*UAS*-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

1. 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 cells in a 96-well plate requires 50ng each of pGL4.35[*luc2P*/9X*GAL4*UAS/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 use 24 hours of 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). The 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). The 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 noninduced 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:

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

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