

Certificate of Analysis

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

Part No.	Size
E137A	20µg

Description: The pGL4.35[*luc2P*/9XGAL4UAS/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.

Part# 9PIE137

Revised 10/16



AF9PIE137 1016E137

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

^(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



Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 2009, 2015, 2016 Promega Corporation. All Rights Reserved.

Bright-Glo and GloResponse are trademarks of Promega Corporation.

GenBank is a registered trademark of the U.S. Department of Health and Human Services.

All specifications are subject to change without prior notice.

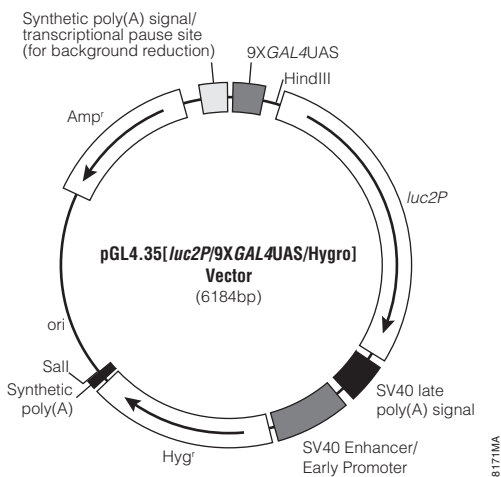
Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Part# 9PIE137
Printed in USA Revised 10/16.



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 (<i>Hyg^r</i>) coding region	2842–3879
Synthetic poly(A) region	3903–3951
<i>ColE1</i> -derived plasmid origin of replication (<i>ori</i>)	4275–4311
Synthetic β -lactamase (<i>Amp^r</i>) 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), 100 μ M solution in ethanol
- luciferase assay system (e.g., Bright-Glo™ Luciferase Assay System, Cat.# E2610; see the 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-ER α 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 (80 μ l/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*/*9XGAL4UAS*/*Hygro*] Vector and a fusion gene containing the Gal4 DNA-binding domain, such as pBIND-ER α 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 10 μ l/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 10 μ l, 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 100 μ M (1:100 dilution). The final dexamethasone concentration in the wells will be 10 μ M. For the pBIND-ER α Vector, dilute 100 μ M 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-ER α Vector.
2. Add 10 μ l 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:

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