

Certificate of Analysis

pBIND-ER α Vector

Part No. Size
E139A 20 μ g

Part# 9PIE139
Revised 12/16



Instructions for use of this product with a compatible cell line can be found in the *GloResponse™ 9XGAL4 UAS-luc2P HEK293 Cell Line Technical Bulletin* #TB552, available at: www.promega.com/protocols

Description: The pBIND-ER α Vector^(a,b) (Cat.# E1390) contains the yeast Gal4 DNA-binding domain (Gal4-DBD) and an estrogen receptor-ligand binding domain (ER-LBD) gene fusion that can induce the transcription of a gene of interest containing an upstream Gal4 Upstream Activator Sequence (UAS) such as that of the pGL4.35[*luc2P/9XGAL4UAS/Hygro*] Vector (Cat.# E1370) when activated by a ligand. The sequence for the estrogen receptor -ligand binding domain contained in this plasmid has a single amino acid difference, which corresponds to amino acid 420 of the GenBank® reference sequence for the full-length estrogen receptor. For information on how the GenBank® sequence performs compared to this sequence, contact Promega Technical Services.

Concentration: 1 μ g/ μ l.

GenBank® Accession Number: GQ229579.

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.



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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

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 1 μ g 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:

R. Wheeler, Quality Assurance

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^(b)U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

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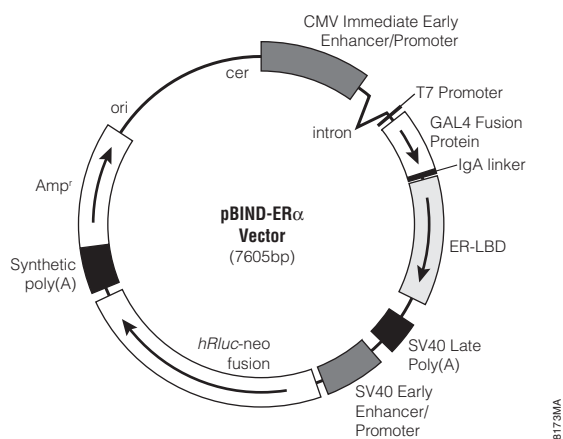
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Part# 9PIE139
Printed in USA. Revised 12/16.



pBIND-ER α Vector Features List and Map:

CMV immediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter	1033–1052
GAL4 DNA binding domain fusion protein	1083–1520
IgA linker	1521–1553
Estrogen receptor ligand binding domain (ER-LBD)	1563–2456
SV40 late poly(A) region	2616–2837
SV40 early enhancer/promoter	2936–3354
<i>hRluc</i> -neomycin fusion protein	3399–5174
Synthetic poly(A) signal/transcriptional pause region	5238–5556
Synthetic β -lactamase (Amp ^r) coding region	5547–6407
<i>ColE1</i> -derived plasmid origin of replication (ori)	6562–6598
Cer	7269–7554



pBIND-ER α Vector Map.

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

Sample Protocol to Determine Induction of the pBIND-ER α Vector with β -Estradiol

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)
- β -estradiol, E2 (Sigma E2758), 100 μ M 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
- pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector (Cat.# E1370)

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 (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 the 96-well plate to be transfected requires 50ng each of pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector and pBIND-ER α Vector (Cat.# E1390). 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 have used a 24-hour 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:** Dilute 100 μ M 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:** 0.1% ethanol in phenol red-free DMEM.
2. Add 10 μ l 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 fold induction as follows:

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