

Certificate of Analysis

pBIND-GR Vector

Part No. Size
E158A 20µg

Part# 9PIE158
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-GR Vector^(a,b) (Cat.# E1581) contains the yeast Gal4 DNA-binding domain (Gal4-DBD) and glucocorticoid receptor-ligand binding domain (GR-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.

Concentration: 1µg/µl.

GenBank® Accession Number: GQ229580.

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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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 noncut 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:

R. Wheeler, Quality Assurance

Promega Corporation

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

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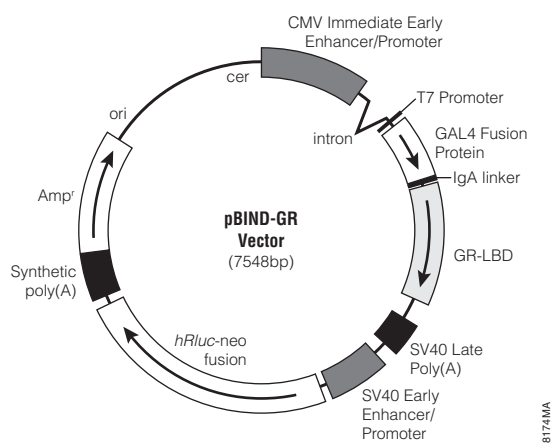
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Part# 9PIE158
Printed in USA. Revised 12/16



pBIND-GR 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 (GAL4-DBD)	1083–1520
IgA linker	1521–1553
Glucocorticoid receptor ligand binding domain (GR-LBD)	1563–2399
SV40 late poly(A) region	2559–2780
SV40 early enhancer/promoter	2879–3297
<i>hRluc</i> -neomycin fusion protein	3342–5117
Synthetic poly(A)/transcriptional pause region	5181–5499
Synthetic β -lactamase (Amp ^r) coding region	5490–6350
<i>ColE1</i> -derived plasmid replication origin	6505–6541
Cer region	7212–7497



pBIND-GR Vector Map.

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

Sample Protocol for Transient Transfection and Induction of the pBIND-GR Vector with Dexamethasone

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 Cat.# D4902), 10mM 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-GR Vector (Cat# E1581). Use a 1:1 ratio of the two vectors. Transfection conditions may require optimization. We routinely add 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 a recovery time of 24 hours 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; prepare 110% of this amount. Use DMEM without phenol red and without FBS for all induction and control solutions.
 - **10X induction solution:** Dilute 10mM dexamethasone solution in phenol red-free DMEM to 100 μ M (1:100 dilution). Final dexamethasone concentration in the wells will be 10 μ M.
 - **10X control solution:** 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. See Section 6.B, Related Products in the *GloResponse™ 9XGAL4UAS-luc2P HEK293 Cell Line* Technical Bulletin, #TB552 for a list of assays.
2. Using the luminescence information, calculate fold induction as follows:

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