# Certificate of Analysis

## pBIND-GR Vector

| Part No. | Size |
|---|---|
| E158A | 20µg |

Part# 9PIE158
6/09

**Instructions for use** of this product with a compatible cell line can be found in the *GloResponse™ 9X*GAL4 *UAS*-luc2P *HEK293 Cell Line Technical Bulletin* #TB552, available at: **www.promega.com/tbs**

**Description:** The pBIND-GR Vector[a,b,c,d] (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*/9X*GAL4*UAS/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.

# 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: J. Stevens

J. Stevens, Quality Assurance

[a]READ THIS FIRST BEFORE OPENING PRODUCT
For research use only. The terms of the limited license conveyed with the purchase of this product are as follows: Researchers may use this product in their own research and they may transfer derivatives to others for such research use provided that at the time of transfer a copy of this label license is given to the recipients and the recipients agree to be bound by the conditions of this 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) 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]The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.
[d]Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

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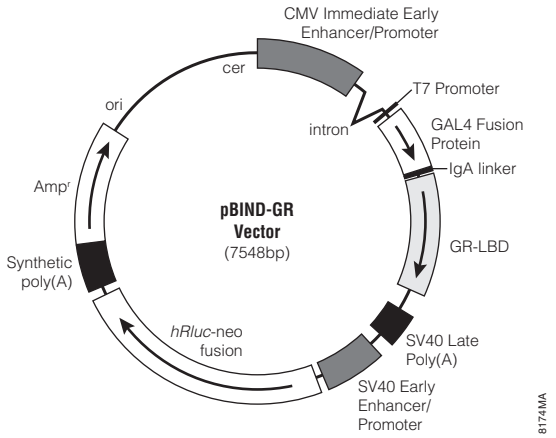
## pBIND-GR Vector Features List and Map:

| | |
|---|---|
| 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 |
| *hRluc*-neomycin fusion protein | 3342–5117 |
| Synthetic poly(A)/transcriptional pause region | 5181–5499 |
| Synthetic β-lactamase (Amp$^r$) coding region | 5490–6350 |
| *Col*E1-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™ 9X*GAL4*UAS*-luc2P *HEK293 Cell Line* Technical Bulletin, #TB552 for additional assays)
- High-efficiency transfection reagent
- HEK293 cells
- pGL4.35[*luc2P*/9X*GAL4*UAS/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 (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*/9X*GAL4*UAS/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 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 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 10µl; 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 100µM (1:100 dilution). Final dexamethasone concentration in the wells will be 10µM.
- **10X control solution:** 1% ethanol in phenol red-free DMEM.

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. See Section 6.B, Related Products in the *GloResponse™ 9X*GAL4*UAS*-luc2P *HEK293 Cell Line* Technical Bulletin, #TB552 for a list of assays.

2. Using the luminescence information, calculate fold induction as follows:

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

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