Certificate of Analysis

pGL4.43[*luc2P*/XRE/Hygro] Vector:

Part No.	Size
E412A	20µg

Part# 9PIE412
Revised 4/18

Description: The pGL4.43[*luc2P*/XRE/Hygro] Vector[a–c] contains three copies of a xenobiotic response element (XRE) that drives transcription of the luciferase reporter gene *luc2P* (*Photinus pyralis*). *luc2P* is a synthetically derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription. The *luc2P* gene contains hPEST, a protein destabilization sequence, which allows luc2P protein levels to respond more quickly than those of luc2 to induction of transcription. The vector backbone contains an ampicillin resistance gene to allow selection in *E. coli* and a gene for hygromycin resistance to allow selection of stably transfected mammalian cell lines.

Concentration: 1µg/µl.

GenBank® Accession Number: JQ858513.

Storage Buffer: The pGL4.43[*luc2P*/XRE/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See the product information label for storage temperature recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the product information label.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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Quality Control Assays

Nuclease Assay: Following incubation of 1µg of the 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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Sequence: The pGL4.43[*luc2P*/XRE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors/**

Signed by:

R. Wheeler, Quality Assurance

Promega Corporation

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[b]U.S. Pat. No. 7,728,118.
[c]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

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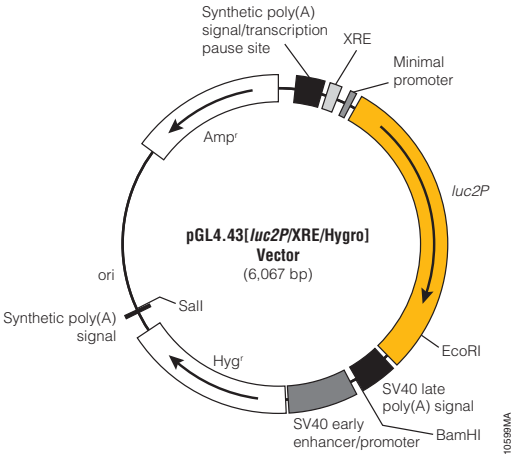
pGL4.43[*luc2P*/XRE/Hygro] Vector Features List and Map:

XRE response element	285–347
Minimal promoter	393–423
luc2P reporter gene	456–2231
SV40 late poly(A) signal	2271–2492
SV40 early enhancer/promoter	2540–2958
Synthetic hygromycin (Hyg^r) coding region	2983–4020
*Col*E1-derived plasmid replication origin	4416
Synthetic β-lactamase (Amp^r) coding region	5207–6067
Synthetic poly(A) signal sequence	4044–4092
Synthetic poly(A) signal/transcriptional pause site	105–258
Reporter Vector primer 3 (RVprimer3) binding region	207–226
Reporter Vector primer 4 (RVprimer4) binding region	4159–4178



Sequence information for the pGL4 Vectors is available online at:
www.promega.com/vectors/

Example Protocol

In this example protocol, the pGL4.43[*luc2P*/XRE/Hygro] Vector is used to measure activation of the XRE in HepG2 cells upon treatment with TCDD. The pGL4.75 Vector (encoding *Renilla* luciferase) is used as a normalization control. In designing such experiments, it is important that the chosen cell type can be transfected efficiently and that it expresses the proper components of the signaling pathway of interest in order to generate the biological response. Protocol optimization may be required for your particular cell type and assay conditions.

Materials to be Supplied by User

- DMEM (Life Technologies Cat.# 11995)
- Complete medium [DMEM supplemented with 10% fetal bovine serum (DMEM/FBS; Life Technologies Cat.# 16000] and 1X NEAA [Life Technologies Cat.# 11140])
- Dulbecco's PBS (DPBS; Life Technologies Cat. # 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- Charcoal-stripped FBS (Life Technologies Cat.# 126776-011)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin; AccuStandard Cat.# D-404N)
- DMSO (Sigma Cat.# D2650)
- Dual-Glo® Luciferase Assay System (Cat.# E2940)
- HepG2 cells
- pGL4.75[*hRluc*/CMV] Vector (Cat.# E6931)

Day 1: Plate Cells

1. Grow HepG2 cells in complete medium (DMEM + 10% FBS + 1X NEAA). Wash twice with DPBS and treat with one volume of 0.05% trypsin-EDTA, followed by four volumes of complete medium.
2. Vigorously resuspend the cells by pipetting and allow cell clumps to settle. Remove the cell suspension from any cell clumps, quantify the cells and dilute in complete medium to 1×10^5 cells/ml.
3. Plate 100µl per well to a solid, white 96-well plate (Corning Cat.# 3917).
4. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Transfection

1. Dilute pGL4.43[*luc2P*/XRE/hygro] and pGL4.75 [*hRluc*/CMV] *Renilla* luciferase vector constructs in a 10:1 mass ratio, respectively, to 12.5ng total DNA/µl in Opti-MEM® I.
2. Add FuGENE® HD to a 4.5:1 lipid:DNA ratio. Mix by pipetting. Incubate at room temperature for 20 minutes.
3. Add 8µl transfection complex per well (100ng DNA/well) and incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 3: Medium Replacement and Cell Treatment

1. Resuspend TCDD to 31.6µM in DMSO. Serially dilute by half logs into DMSO to give concentrated stock solutions (316X). Dilute these 31.6-fold into Opti-MEM® I to give 10X stocks.
2. Remove existing medium from cells and replace with 72µl of DMEM + 0.5% charcoal-stripped FBS per well.
3. Add 8µl of the 10X TCDD dilutions and incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 4: Luminescence Measurement

1. Remove plates from the 37°C, 5% CO_2 incubator and allow to cool to room temperature for approximately 15 minutes.
2. Add 80µl of the Dual-Glo® Luciferase Assay System detection reagents and measure luminescence following the recommended protocol (Refer to the Dual-Glo® Luciferase Assay System Technical Manual, #TM058 for details).

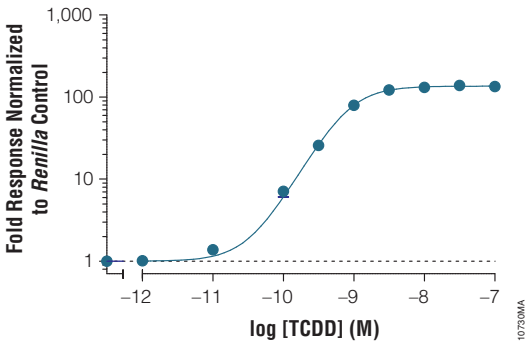


Figure 1. Representative data for pGL4.43[*luc2P*/XRE/Hygro] in HepG2 cells upon stimulation with TCDD. HepG2 cells were transiently transfected with pGL4.43[*luc2P*/XRE/Hygro] and pGL4.75 and assayed in 96-well format after 24 hours stimulation with TCDD as indicated in the protocol. Firefly luciferase luminescence normalized to the *Renilla* luciferase control is shown, with error bars indicating the S.E.M. for six replicates. Luminescence was detected after addition of Dual-Glo® reagents, using a GloMax® 96 instrument with a 0.5 second integration time.

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