Certificate of Analysis

pGL4.40[*luc2P*/MRE/Hygro] Vector:

Part No.	Size
E413A	20µg

Description: The pGL4.40[*luc2P*/MRE/Hygro] Vector[a–e] contains five copies of a metal response element (MRE) 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: JQ858515.

Storage Buffer: The pGL4.40[*luc2P*/MRE/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.

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 pGL4.40[*luc2P*/MRE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors/**

Signed by: J. Stevens

J. Stevens, Quality Assurance

[a]READ THIS FIRST BEFORE OPENING PRODUCT.
The sale of this product and its use are subject to the terms of a limited use label license, the full text of which is available at: **www.promega.com/LULL**. That text must be read by the purchaser prior to opening this product to determine whether the purchaser agrees that all use of the product shall be in accordance with the license terms. If the purchaser is not willing to accept the terms of the limited use label license, Promega is willing to accept the return of the unused product and provide the purchaser with a full refund. However, if the product is opened for any reason, then the purchaser agrees to be bound by the terms of the limited use label license.
[b]U.S. Pat. No. 7,728,118.
[c]U.S. Pat. No. 5,670,356.
[d]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.
[e]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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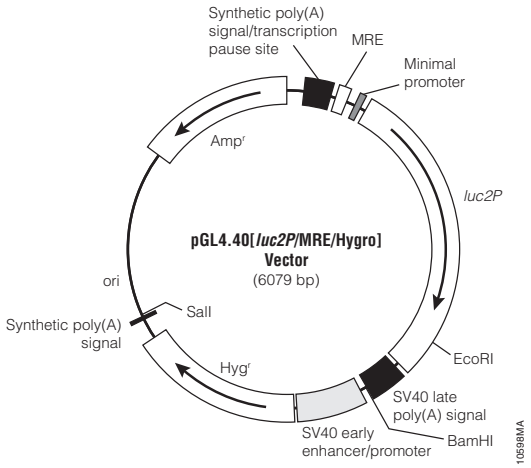
pGL4.40[*luc2P*/MRE/Hygro] Vector Features List and Map:

MRE response element	285–359
Minimal promoter	405–435
luc2P reporter gene	468–2243
SV40 late poly(A) signal	2283–2504
SV40 early enhancer/promoter	2552–2970
Synthetic hygromycin (Hyg^r) coding region	2995–4032
*Col*E1-derived plasmid replication origin	4428
Synthetic β-lactamase (Amp^r) coding region	5219–6079
Synthetic poly(A) signal sequence	4056–4104
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	4171–4190



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

Example Protocol

In this example protocol, the pGL4.40[*luc2P*/MRE/Hygro] vector is used to measure activation of the MRE in HepG2 cells upon treatment with Zinc Sulfate. 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)
- $ZnSO_4$ (Sigma Cat.# Z4750)
- 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 pGL4.40[*luc2P*/MRE/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

1. Remove existing medium from cells and replace with 72µl of DMEM + 0.5% charcoal-stripped FBS per well.
2. Incubate for 24 hours in a 37°C, 5% CO_2 incubator.

Day 4: Cell Treatment and Luminescence Measurement

1. Serially dilute a 10mM aqueous stock of $ZnSO_4$ into water to give 10X stocks.
2. Add 8µl of the 10X dilutions of $ZnSO_4$ and incubate for 6 hours in a 37°C, 5% CO_2 incubator.
3. Remove plates from the 37°C, 5% CO_2 incubator and allow to cool to room temperature for approximately 15 minutes.
4. 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).

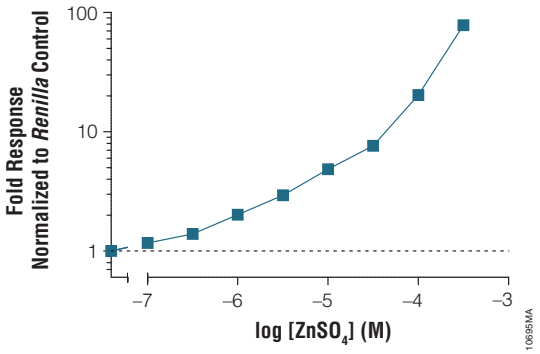


Figure 1. Representative data for pGL4.40[luc2P/MRE/Hygro] in HepG2 cells upon stimulation with $ZnSO_4$. HepG2 cells were transiently transfected with pGL4.40[*luc2P*/MRE/Hygro] and pGL4.75 and assayed in 96-well format after six hours stimulation with $ZnSO_4$ as indicated in the protocol. Firefly luciferase luminescence normalized to the *Renilla* luciferase control is shown. Error bars indicate 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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