Certificate of Analysis

pGL4.49[*luc2P*/TCF-LEF RE/Hygro] Vector:

Part No.	Size
E461A	20µg

Description: The pGL4.49[*luc2P*/TCF-LEF RE/Hygro] Vector[a–e] contains eight copies of a TCF-LEF response element (TCF-LEF RE) 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: JX099537.

Storage Buffer: The pGL4.49[*luc2P*/TCF-LEF RE/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 Notes:

This plasmid may show instability when propagated in certain strains of *E. coli*, losing one or more of the TCF-LEF RE repeats upstream of the minimal promoter. We recommend propagating this strain in TOP10 cells (Life Technologies Cat.# C4040-03) at 37°C.

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 pGL4.49[*luc2P*/TCF-LEF RE/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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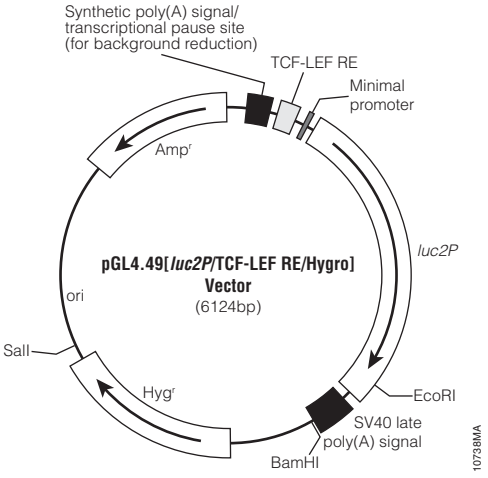
pGL4.49[*luc2P*/TCF-LEF RE/Hygro] Vector Features List and Map:

TCF-LEF response element	285–404
Minimal promoter	450–480
luc2P reporter gene	513–2288
SV40 late poly(A) signal	2328–2549
SV40 early enhancer/promoter	2597–3015
Synthetic hygromycin (Hyg^r) coding region	3040–4077
*Col*E1-derived plasmid replication origin	4473
Synthetic β-lactamase (Amp^r) coding region	5264–6124
Synthetic poly(A) signal sequence	4101–4149
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	4216–4235



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

Example Protocol

In this example protocol, the pGL4.49 [*luc2P*/TCF-LEF RE/Hygro] vector is used to measure activation of the TCF-LEF RE in HEK293 cells upon treatment with mWnt3a or LiCl. 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

- Dulbecco's PBS (DPBS; Life Technologies Cat.# 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- 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])
- Charcoal-stripped FBS (Life Technologies Cat.# 126776-011)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- mWnt3a (R&D Systems Cat.# 1324-WN)
- BSA (Proliant cat# 68700)
- LiCl (Sigma Cat.# 40113)
- ONE-Glo™ Luciferase Assay System (Cat.# E6110)
- HEK293 cells

Day 1: Reverse Transfection

Preparation of Cells

1. Grow HEK293 cells in complete medium [DMEM + 10% FBS + 1X NEAA]. Wash with DPBS and treat with one volume of 0.05% trypsin-EDTA. Resuspend cells in four volumes of complete medium.
2. Quantify cells and dilute in complete medium to 1.5×10^5 cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.49[*luc2P*/TCF-LEF RE/Hygro] Vector to 10ng total DNA/µl in Opti-MEM® I.
2. Add FuGENE® HD to a 3:1 lipid:DNA ratio. Mix by pipetting. Incubate at room temperature for 30 minutes.
3. Dilute lipid:DNA mixture 20-fold with 1.5×10^5 cells/ml cell suspension. Mix by inversion.
4. Plate 100µl per well into a solid, white 96-well plate (Corning Cat.# 3917).
5. Incubate for 18 hours in a 37°C, 5% CO_2 incubator.

Day 2: Medium Replacement and Cell Treatment

1. Resuspend mWnt3a to 100µg/ml in DPBS + 1 mg/ml BSA. Dilute mWnt3a to 3µg/ml and then serially dilute into PBS/BSA to give a range of 10X stock solutions. Serially dilute 1M LiCl into water to give a range of 10X stock solutions.
2. Remove existing medium from cells and replace with 90µl of DMEM + 0.5% charcoal-stripped FBS per well.
3. Add 10µl of the 10X dilutions of mWnt3a or LiCl.
4. Incubate for 8 hours in a 37°C, 5% CO_2 incubator.

Day 3: Luminescence Measurement

1. Remove plates from the incubator and allow them to cool to room temperature for approximately 15 minutes.
4. Add ONE-Glo™ Luciferase Assay System detection reagent, and measure luminescence following the recommended protocol (refer to the *ONE-Glo™ Luciferase Assay System Technical Manual*, #TM292 for details).

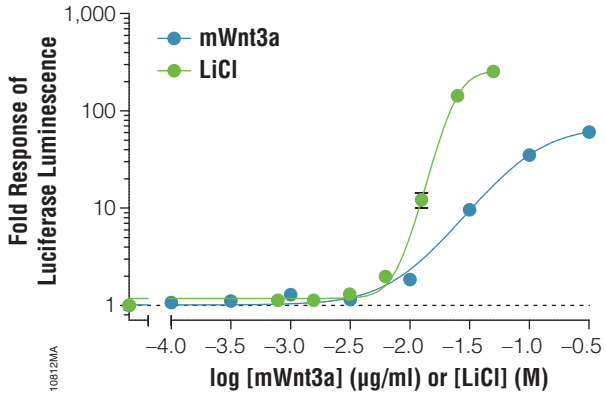


Figure 1. Representative data for pGL4.49[*luc2P*/TCF-LEF RE/Hygro] in HEK293 cells upon stimulation with mWnt3a or LiCl. HEK293 cells were transiently transfected with pGL4.49[*luc2P*/TCF-LEF RE/Hygro] and assayed in 96-well format after 8 hours stimulation with mWnt3a or LiCl as indicated in the protocol. Firefly luciferase luminescence normalized to untreated cells is shown, with error bars indicating S.E.M. for three replicates. Luminescence was detected after addition of ONE-Glo™ reagent, using a GloMax® 96 instrument with a 0.5 second integration time.

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