Certificate of Analysis

pGL4.48[*luc2P*/SBE/Hygro] Vector:

Part No.	Size
E367A	20µg

Description: The pGL4.48[*luc2P*/SBE/Hygro] Vector[a–f] contains three copies of a Smad binding element (SBE) 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: JQ858517.

Storage Buffer: The pGL4.48[*luc2P*/SBE/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 pGL4.48[*luc2P*/SBE/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]Licensed under U.S. Pat. No. 6,100,032.
[c]U.S. Pat. No. 7,728,118.
[d]U.S. Pat. No. 5,670,356.
[e]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.
[f]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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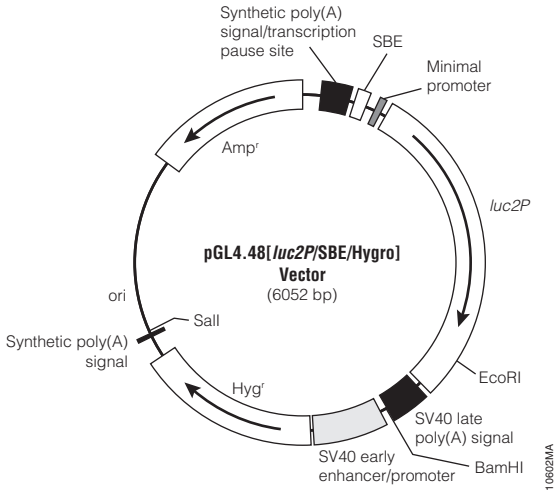
pGL4.48[*luc2P*/SBE/Hygro] Vector Features List and Map:

SBE response element	285–332
Minimal promoter	378–408
luc2P reporter gene	441–2216
SV40 late poly(A) signal	2256–2477
SV40 early enhancer/promoter	2525–2943
Synthetic hygromycin (Hyg^r) coding region	2968–4005
Co/E1-derived plasmid replication origin	4401
Synthetic β-lactamase (Amp^r) coding region	5192–6052
Synthetic poly(A) signal sequence	4029–4077
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	4144–4163



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

Example Protocol

In this example protocol, the pGL4.48[*luc2P*/SBE/Hygro] vector is used to measure activation of the SBE in HEK293 cells upon treatment with TGF-β1. 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% Trypsin-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])
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- Human TGF-β1 (Sigma Cat.# T7039-2UG)
- BSA (Sigma Cat.# B4287)
- ONE-Glo™ Luciferase Assay System (Cat.# E6120)
- 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. Pellet the cells by centrifugation at 233 x *g* for 5 minutes in a swinging-bucket rotor. Resuspend in complete medium at a concentration of 2×10^5 cells/ml.

Preparation of Lipid:DNA Mixture

1. Dilute pGL4.48[*luc2P*/SBE/Hygro] DNA to 10ng 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 2×10^5 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: Cell Treatment and Luminescence Measurement

1. Resuspend human TGF-β1 (hTGF-β1) to 50µg/ml in water. Dilute 50X into DPBS + 2 mg/ml BSA to give a 1µg/ml solution, and then serially dilute into the same buffer to give 10X stocks.
2. Add 10µl of the 10X dilutions of hTGF-β1 to each well and incubate for 3 hours in a in a 37°C, 5% CO_2 incubator.
3. Remove plates from the 37°C, 5% CO_2 incubator and allow them to cool to room temperature for approximately 15 minutes.
4. Add ONE-Glo™ Luciferase Assay System detection reagent to each well and measure luminescence following the recommended protocol (Refer to the ONE-Glo™ Luciferase Assay System Technical Manual, #TM292 for details).

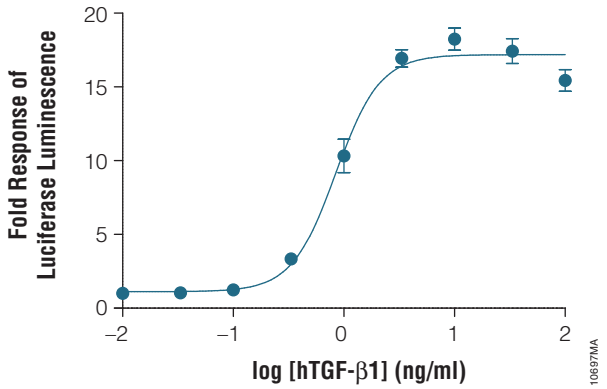


Figure 1. Representative data for pGL4.48[*luc2P*/SBE/Hygro] in HEK293 cells upon stimulation with hTGF-β1. HEK293 cells were transiently transfected with pGL4.48[*luc2P*/SBE/Hygro] and assayed in 96-well format after three hours stimulation with hTGF-β1 as indicated in the protocol. Firefly luciferase 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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