Certificate of Analysis

pGL4.29[*luc2P*/CRE/Hygro] Vector:

Part No.	Size
E847A	20µg

> ℹ️ **Instructions for use** of this product can be found in the *pGL4 Luciferase Reporter Vectors Technical Manual #TM259*, available online at: **www.promega.com/protocols**

Description: The pGL4.29[*luc2P*/CRE/Hygro] Vector[a–f] contains a cAMP response element (CRE) that drives the 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. The protein encoded by *luc2P* responds more quickly than the protein encoded by the *luc2* gene upon induction. The vector backbone contains an ampicillin resistance gene to allow selection in *E. coli* and a mammalian selectable marker for hygromycin resistance.

See the *pGL4 Luciferase Reporter Vectors Technical Manual #TM259* for more information.

Concentration: 1µg/µl.

GenBank® Accession Number: DQ904461.

Storage Buffer: The pGL4.29[*luc2P*/CRE/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. These fluctuations can greatly alter product stability. 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 digest buffer B at 37°C for 16 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$ at pH 7.4.

Sequence: The pGL4.29[*luc2P*/CRE/Hygro] Vector has been completely sequenced and has 100% identity with the published sequence, available at: **www.promega.com/vectors**

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[b]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

[c]Patent Pending.

[d]U.S. Pat. No. 7,728,118.

Signed by: *R. Wheeler signature*

R. Wheeler, Quality Assurance

pGL4.29[*luc2P*/CRE/Hygro] Vector Features List and Map:

cAMP response element (CRE)	33–119
Minimal promoter	152–182
luc2P reporter gene	215–1990
SV40 late poly(A) region	2030–2251
SV40 early enhancer/promoter	2299–2717
Synthetic hygromycin (Hyg^r) coding region	2742–3779
Synthetic poly(A) region	3803–3851
Reporter Vector primer 4 (RVprimer4) binding region	3918–3937
*Col*E1-derived plasmid replication origin	4175
Synthetic β-lactamase (Amp^r) coding region	4966–5826
Synthetic poly(A) signal/transcriptional pause region	5931–6084
Reporter Vector primer 3 (RVprimer3) binding region	6033–6052
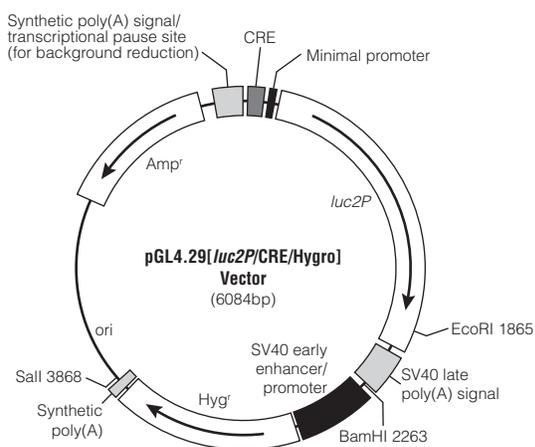
pGL4.29[*luc2P*/CRE/Hygro] Vector Map.

Sequence information and restriction enzyme tables for the pGL4 Vectors are available online at: **www.promega.com/vectors**

For more information see the *pGL4 Luciferase Reporter Vectors Technical Manual*, #TM259, available online at: **www.promega.com/protocols**

Sample Protocol to Determine Luciferase Induction in HEK 293 Cells Transfected with pGL4.29[*luc2P*/CRE/Hygro] Vector

Materials to be Supplied by User

- Dulbecco's PBS (DPBS)
- 0.05% (w/v) trypsin in DPBS
- DMEM supplemented with 10% fetal bovine serum (DMEM/FBS)
- *Trans*IT®-LT1 (Mirus Part# MIR2304)
- DMSO
- 100mM forskolin in DMSO
- Bright-Glo™ Luciferase Assay System (Cat.# E2610)
- HEK 293 cells

Day 1: Plate Cells

1. Grow HEK 293 cells in DMEM/FBS to approximately 75% confluency.

2. Harvest cells via trypsinization. Remove the DMEM/FBS, wash the cells with DPBS and add the trypsin/DPBS (1X volume). After 2 minutes, add a 4X volume of DMEM/FBS, collect the cell suspension and pellet the cells by centrifugation. Aspirate the supernatant and resuspend in DMEM/FBS at a concentration of 10,000 viable cells/90µl DMEM/FBS.

3. Dispense 90µl of the cell suspension into the wells of a 96-well plate. Plate enough wells to perform each test condition in triplicate.

4. Cover the plate and place it in a tissue culture incubator at 37°C overnight (or for 24 hours).

Day 2: Transfect Cells

1. Prepare the DNA transfection master mix. Each well of 96-well plate to be transfected requires 10µl DMEM, 0.3µl *Trans*IT®-LT1 and 0.1µg pGL4.29[*luc2P*/CRE/Hygro] plasmid DNA. To prepare the master mix, calculate the total number of wells that will be transfected and prepare 110% of this amount. It is recommended that at least 10 wells of master mix be prepared.

- For each well, mix 10µl DMEM and 0.3µl *Trans*IT®-LT1 in a microcentrifuge tube, briefly vortex at maximum setting, and incubate at room temperature for 15 minutes.

- For each well to be transfected, add 0.1µg of pGL4.29[*luc2P*/CRE/Hygro] vector to the DMEM/*Trans*IT®-LT1, vortex briefly and incubate at room temperature for 15 minutes.

2. Add 10µl of master mix to each well that is to be transfected.

3. Cover the plate and place it in a tissue culture incubator at 37°C overnight (or for 24 hours).

Day 3: Induce Transfected Cells and Measure Luciferase Activity

1. Prepare 10X induction and 10X control solutions. Calculate the volume of 10X induction and 10X control solution by multiplying the number of wells needed for each solution by 11µl and prepare 110% of this amount.

- 10X induction solution: Dilute 100mM stock forskolin solution to 1mM (1:100) in DMEM.

- 10X control solution: Dilute DMSO 1:100 in DMEM.

2. Add 11µl of 10X induction solution to the cells to be induced and 11µl of 10X control solution to the control noninduced cells.

3. Return the plate to the tissue culture incubator and induce for 5 hours.

4. Analyze luciferase activity using the Bright-Glo™ Luciferase Assay System as described in Technical Manual #TM052.

5. Calculate the fold induction as follows:

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