Certificate of Analysis

pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector:

Part No.	Size
E848A	20µg

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**

Part# 9PIE848
Revised 9/16

Description: The pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector[a–f] contains an NFAT response element (NFAT-RE) 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: DQ904462.

Storage Buffer: The pGL4.30[*luc2P*/NFAT-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. 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 pGL4.30[*luc2P*/NFAT-RE/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

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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.

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pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector Features List and Map:

NFAT response element	33–122
Minimal promoter	136–166
luc2P reporter gene	228–2003
SV40 late poly(A) signal	2043–2264
SV40 early enhancer/promoter	2312–2730
Synthetic hygromycin (Hyg^r) coding region	2755–3792
Synthetic poly(A) signal	3816–3864
Reporter Vector primer 4 (RVprimer4) binding region	3931–3950
*Col*E1-derived plasmid replication origin	4188
Synthetic β-lactamase (Amp^r) coding region	4979–5839
Synthetic poly(A) signal/transcriptional pause site	5944–6097
Reporter Vector primer 3 (RVprimer3) binding region	6046–6065

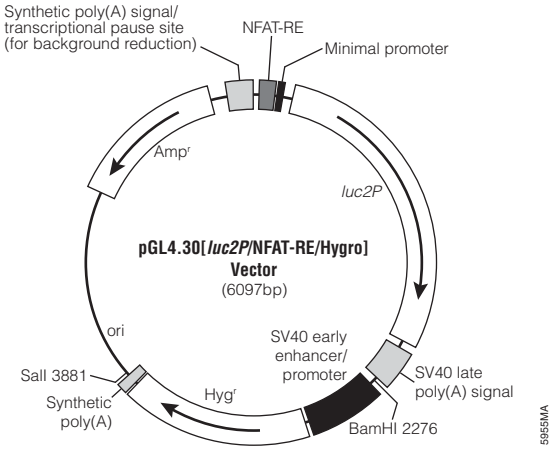

Figure 1. Vector map.

Sample Protocol to Determine Luciferase Induction by Ionomycin/PMA in HEK 293 Cells Transfected with pGL4.30[*luc2P*/NFAT-RE/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
- 1mM ionomycin in DMSO
- 1mg/ml phorbol 12-myristate 13-acetate (PMA) 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 the cells via trypsinization. Remove the DMEM/FBS, wash the cells with DPBS and add the trypsin/DPBS (1X volume). After 2 minutes, add 4X volume of DMEM/FBS, collect the cell suspension and pellet the cells by centrifugation. Aspirate supernatant and resuspend in DMEM/FBS at a concentration of 20,000 viable cells/90µl DMEM/FBS.
3. Dispense 90µl of the cell suspension to 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 pGL4.30[*luc2P*/NFAT-RE/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 pGL4.30[*luc2P*/NFAT-RE/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 1X induction and 1X control solutions. Calculate the volume of 1X induction and 1X control solution by multiplying the number of wells needed for each solution by 100µl and prepare 110% of this amount.
   - 1X induction solution: Dilute 1mM stock ionomycin solution to 1µM (1:1,000) and 1mg/ml stock solution of PMA to 10ng/ml (1:100,000) in DMEM/FBS.
   - 1X control solution: Dilute DMSO 1:1,000 in DMEM/FBS.
2. Remove medium from wells that will be treated with either 1X induction or 1X control solutions.
3. Add 100µl of 1X induction solution to the cells to be induced and 100µl of 1X control solution to the control noninduced cells.
4. Return the plate to the tissue culture incubator and induce for 17 hours.

   For different applications in other cell backgrounds or using other stimulating drugs, we suggest that you optimize assay induction time within a range of 5–24 hours and choose an induction time that gives the optimal assay response.

Day 4: Analyze Luciferase Activity

1. Analyze luciferase activity using the Bright-Glo™ Luciferase Assay System as described in Technical Manual #TM052.
2. Calculate the fold induction as follows:

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

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