Certificate of Analysis

pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector:

Part No.	Size
N111A	20µg

Part# 9PIN111
Revised 9/16

Instructions for use of this product can be found in the Nano-Glo® Luciferase Assay System Technical Manual #TM369, available online at:
www.promega.com/protocols

AF9PIN111 0916N111

Description: The pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector[a,b] contains 5 copies of an NF-κB response element (NF-κB-RE) that drives transcription of a destabilized form of NanoLuc® luciferase, an engineered 23.3kDa luciferase fusion protein. The NlucP reporter consists of NanoLuc® luciferase with a C-terminal fusion to PEST, a protein destabilization domain, which responds more quickly and with greater magnitude to changes in transcriptional activity than unmodified NanoLuc® luciferase. The *NlucP* gene is codon optimized for expression in mammalian cells, and all pNL vectors and *Nluc* genes have minimal consensus transcription factor-binding sites to reduce anomalous expression. The vector contains an ampicillin-resistance gene for selection in *E. coli* and a selectable marker for hygromycin resistance in mammalian cells. All forms of NanoLuc® luciferases should be used with the optimized substrate, furimazine, found in the Nano-Glo® Assay Reagents.

Concentration: 1µg/µl.

GenBank® Accession Number: JQ513377.

Storage Buffer: pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See the product information label for storage recommendations and expiration date.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of the vector as determined by agarose gel electrophoresis.

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

Functional Assays

Identity Assay: The vector was sequenced completely and has 100% identity with the published sequence available at: www.promega.com/vectors

Restriction Digestion: The functional purity of vector DNA is verified by successful digestion with restriction enzymes at the optimal temperature for one hour. Samples are examined by agarose gel electrophoresis to compare cut and uncut vector DNA with marker DNA.

PLEASE SEE THE BACK OF THIS PAGE
FOR PROTOCOL INFORMATION.

Signed by:

R. Wheeler, Quality Assurance

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

Part# 9PIN111
Printed in USA. Revised 9/16

pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector Features List and Circle Map

NF-κB response elements	33–84
Minimal promoter	117–147
NlucP (NanoLuc®-PEST) reporter gene	180–818
SV40 late poly(A) signal	858–1079
SV40 early enhancer/promoter	1127–1545
Synthetic hygromycin (Hyg^r) coding region	1570–2607
Synthetic poly(A) signal	2631–2679
Reporter Vector primer 4 (RVprimer4) binding region	2746–2765
*Co*lE1-derived plasmid replication origin	3003
Synthetic β-lactamase (Amp^r) coding region	3794–4654
Synthetic poly(A) signal/transcriptional pause site	4759–4912
Reporter Vector primer 3 (RVprimer3) binding region	4861–4880

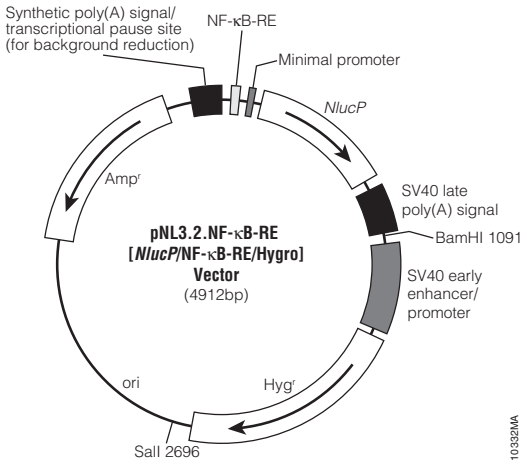

Figure 1. pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector map and sequence reference points.

Sequence information and vector maps for the NanoLuc® Vectors are available online at: **www.promega.com/vectors**

Further information on the use of NanoLuc® Vectors is available in Technical Manual #TM369, which is available online at: **www.promega.com/protocols**

Sample Protocol to Determine Induction of Luciferase by TNFα in HEK 293 Cells Transfected with the pNL3.2.NF-κB-RE[*NlucP*/NF-κB-RE/Hygro] Vector

Materials to be Supplied by User

- Dulbecco's PBS (DPBS)
- 0.05% (w/v) trypsin in DPBS
- DMEM
- DMEM supplemented with 10% fetal bovine serum (DMEM/FBS)
- Tumor necrosis factor-α (Sigma T0157), 10µg/ml solution in PBS containing 1mg/ml BSA
- Nano-Glo® Luciferase Assay System (Cat.# N1110)
- HEK 293 cells
- Transfection reagent (e.g., FuGENE® HD Transfection Reagent, Cat.# E2311)

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. We have routinely used a concentration of 15,000 viable cells/100µl DMEM/FBS.
3. Dispense 100µl 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. Transfect the cells using a high-efficiency transfection reagent (e.g., FuGENE® HD Transfection Reagent, Cat.# E2311). Transfection conditions may require optimization.
2. Cover the plate and place it in a tissue culture incubator at 37°C overnight or as needed for cell recovery depending on the transfection method used. We have used 24 hours recovery time for lipid-mediated transfections.

Day 3: Induce Transfected Cells

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 10µg/ml TNFα solution to 20ng/ml in DMEM/FBS. Final TNFα concentration will be 20ng/ml.
   - 1X control solution: DMEM/FBS.
2. Remove media from wells that will be treated with either 1X induction solution or 1X control solution.
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 5 hours.
5. Analyze luciferase activity using the Nano-Glo® Luciferase Assay System (Cat.# N1110, Technical Manual #TM369).
6. Calculate the fold induction as follows:

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

Part# 9PIN111
Printed in USA. Revised 9/16