Certificate of Analysis

pNLF1-NRF2 [CMV/neo] Vector:

Part No.	Size
N139A	20µg

Part# 9PIN139

Revised 9/16

AF9PIN139 0916N139

Description: The pNLF1-NRF2 [CMV/neo] Vector[a,b] encodes an NRF2-NanoLuc® fusion protein under control of a mammalian (CMV) promoter. NanoLuc® luciferase is engineered and optimized for extreme brightness, small size and robust thermostability. The vector backbone contains a neomycin phosphotransferase gene to allow selection with kanamycin in *E. coli* or with neomycin in mammalian cell lines.

This product also includes a pKEAP1 expression vector (pKEAP1) for proper regulation of intracellular NRF2 levels. The pKEAP Vector encodes a hygromycin-resistance gene for selection in mammalian cells, as well as an ampicillin-resistance gene for selection in bacteria. Transfection Carrier DNA has also been included to allow for diluted expression of the NRF2-NanoLuc® luciferase or KEAP1.

Concentration: 1µg/µl.

GenBank® Accession Numbers: KF853602 for the pNLF1-NRF2 [CMV/neo] Vector; KF853604 for the pKEAP1 Vector.

Storage Buffer: The pNLF1-NRF2 [CMV/neo] Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

Storage Conditions: See the Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See label for expiration date.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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.

Endotoxin Concentration: Endotoxin Units (EU) are obtained using Limulus amoebocyte lysate testing. The specification is <100EU/mg of plasmid DNA.

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

Functional Assays

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

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

Signed by:

R. Wheeler, Quality Assurance

Promega

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# 9PIN139
Printed in USA. Revised 9/16

Promega

Usage Information

pNLF1-NRF2 [CMV/neo] Vector Features

The following features are present in the vector based on nucleotide sequence.

CMV immediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
NRF2 coding region	1065–2879
Linker	2889–2900
NanoLuc® protein coding region	2901–3410
SV40 late poly(A) region	3547–3768
SV40 early enhancer/promoter	3867–4279
EM7 bacterial promoter	4293–4359
Neomycin phosphotransferase coding region	4373–5167
Synthetic poly(A) signal	5231–5279
ColE1-derived plasmid replication origin	5515–5551

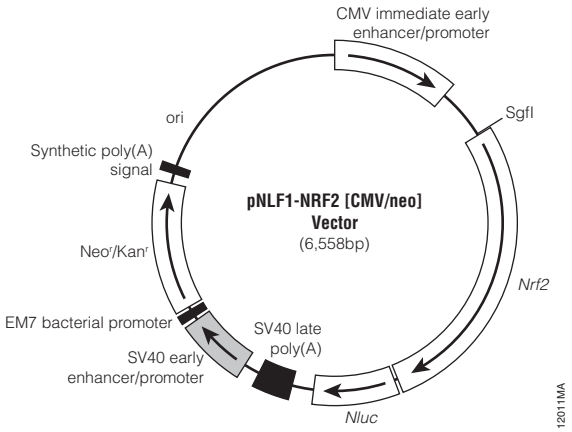

Figure 1. pNLF1-NRF2 [CMV/neo] Vector map.

Sample Protocol

In this sample protocol, pNLF1-NRF2 [CMV/neo] Vector is used to measure activation of reactive oxygen species (ROS) signaling in HCT-116 cells upon treatment with chemical insults, such as D,L-Sulforaphane. The transfection carrier DNA and pKEAP1 DNA are used to properly control intracellular levels of NRF2-NanoLuc® fusion proteins. This protocol is designed for use with HCT-116 cells. If other cell types are used, protocol optimization may be warranted for optimal transfection and ROS response.

Materials to be Supplied by User

- Complete medium (McCoy's 5A; Life Technologies Cat.# 16600) + 10% FBS (Life Technologies Cat.# 16000)
- Dulbecco's PBS (DPBS; Life Technologies Cat. # 14190)
- 0.05% Tryspin-EDTA (Life Technologies Cat.# 25300)
- Opti-MEM® I (Life Technologies Cat.# 31985)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- D,L-Sulforaphane (Sigma Cat.#S4441); Prepare 1,000X D,L-Sulforaphane at 100mM in pure DMSO.
- DMSO (Sigma Cat.# D2650)
- Nano-Glo® Luciferase Assay System (Cat.# N1110)
- white 96-well plates (Corning Cat.# 3917)
- HCT-116 Cells

Day 1: Plate Cells

1. Grow HCT-116 cells in complete medium (McCoy's 5A + 10% FBS + 1X sodium pyruvate). Wash with DPBS and treat with one volume of 0.05% trypsin- EDTA. Resuspend the cells in at least four volumes of complete medium.
2. Quantify the cells and dilute to 5×10^4 cells/ml in complete medium.
3. Plate 90µl of cells per well to a solid white 96-well plate.
4. Incubate for 18–24 hours in a 37°C, 5% CO_2 incubator.

Day 2: Transfection

1. Prepare a 10µg/ml solution of total DNA in Opti-MEM® I. **Note:** To ensure accuracy of DNA concentrations, it may be necessary to use serial dilution steps in a DNA-compatible buffer.

a. Add pKEAP1 DNA to 9.9ng/µl. **Note:** To optimize the expression level of KEAP1, dilute this plasmid into Transfection Carrier DNA (provided).

b. Add pNLF1-NRF2 [CMV/Neo] Vector DNA to 0.1ng total DNA/µl in Opti-MEM® I (including 9.9ng/µl pKEAP1). **Note:** To optimize assay results, adjust the ratio of pKEAP1 DNA to pNLF1-NRF2 [CMV/neo] Vector DNA.

2. Add FuGENE® HD to a 3:1 lipid to DNA ratio (µl FuGENE®HD to µg DNA). Mix gently by inversion several times. Incubate at room temperature for 20 minutes.
3. Add 10µl of transfection complex per well (100ng DNA/well) and incubate for 16–20 hours in a 37°C, 5% CO_2 incubator.

Day 3: Treat Cells

1. Prepare 10X stock solutions of serially diluted positive control agonist D,L-Sulforaphane, beginning at 1mM. Prepare a 10-point serial dilution, with a 3-fold dilution at each step, including a vehicle control. Do not exceed 2% DMSO in the 10X stock, so that the final concentration of DMSO on cells does not exceed 0.2%.
2. Prepare 10X stock solutions of serial-diluted test compounds/unknowns. Do not exceed 2% DMSO in the 10X stock.
3. Add 10µl of the 10X controls or test compounds to each well of the 96-well plate, and incubate 2–4 hours in a 37°C, 5% CO_2 incubator.

Measure Luminescence

1. Remove plates from the 37°C, 5% CO_2 incubator and allow to cool to room temperature for approximately 15 minutes.
2. Add 100µl per well of the Nano-Glo® Luciferase Assay System detection reagents and measure luminescence following the recommended protocol (see the *Nano-Glo® Luciferase Assay System Technical Manual* #TM369 for details).

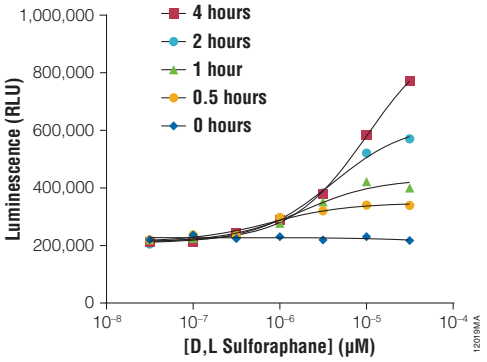

Figure 2. Representative data for the pNLF1-NRF2 [CMV/neo] Vector in HCT-116 cells upon stimulation with D,L-Sulforaphane. HCT-116 cells were transiently transfected with the pNLF1-NRF2 [CMV/Neo] Vector and pKEAP1 DNA as described above. Cells were stimulated with D,L-Sulforaphane for the time indicated and assayed in 96-well format. Luminescence was detected after addition of Nano-Glo® Reagent, using a GloMax® 96 Instrument with a 0.5-second integration time.

Part# 9PIN139
Printed in USA. Revised 9/16