6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

NF-κB Luciferase Reporter Lentivirus

Catalog #: 79564

Product Description

The NF-κB Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the NF-κB signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of NF-κB signaling pathway in the transduced target cells
- Generation of NF-κB Luciferase Reporter stable cell line

Formulation

The lentiviruses were produced from HEK293T cells in the medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of NF-κB luciferase reporter lentivirus at a titer 1 x 10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal.

Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

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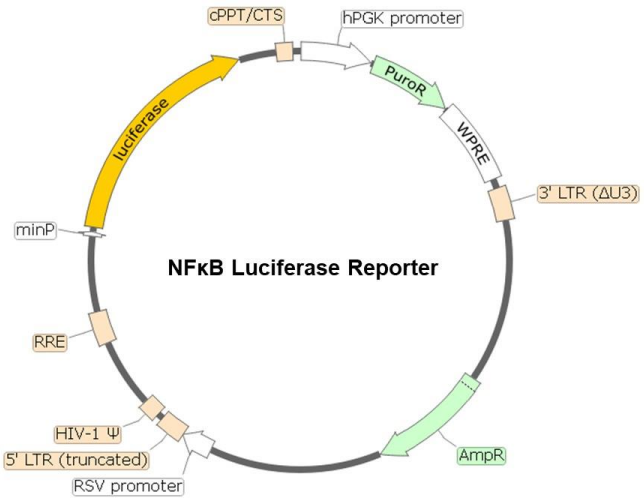

Figure 1. Schematic of the lenti-vector used to generate the NF-κB luciferase reporter lentivirus

Materials Required but Not Supplied

- TNFα (Sigma, #T0157-10UG)
- HEK293 growth medium or use
  Thaw Medium 9 (BPS Bioscience #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using NF-κB luciferase reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirement. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

1. Day 1: Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 9 (BPS Bioscience #79665). Incubate cells at 37°C with 5% CO_2 overnight.

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2. Day 2: To each well add 10 µl of NF-κB luciferase reporter lentivirus. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

   Alternatively, seeding cells and the transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Thaw Medium 9 (BPS Bioscience #79665) to each well.

   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

4. On the morning of Day 5, prepare diluted TNFα in Thaw Medium 9 (BPS Bioscience #79665). Add 10 µl of diluted TNFα to the TNFα-stimulated wells. Add 10 µl of Thaw Medium 9 (BPS Bioscience #79665) to the unstimulated control wells (for measuring the uninduced level of NF-κB reporter activity).

5. Incubate at 37°C with 5% CO_2 for 5-6 hours.

6. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes:

1. To generate the NF-κB luciferase reporter stable cell line, on day 4 remove Thaw Medium 9 (BPS Bioscience #79665) and replaced it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

   1) Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.

   2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

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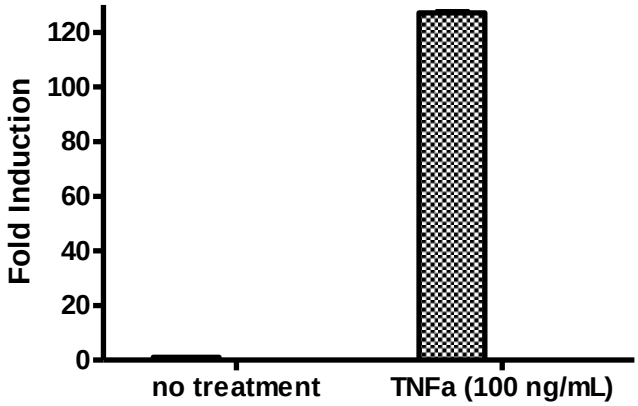

Figure 2. NF-κB luciferase reporter activity stimulated by TNFα in HEK293 cells. Appropriate 10,000 HEK293 cells/well were transduced with 100,000 TU/well NF-κB luciferase reporter lentivirus. After 48 hours of transduction, medium was changed to HEK growth medium. After 66 hours of transduction, cells were treated with 100 ng/mL of TNFα for ~ 6 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without TNFα treatment.

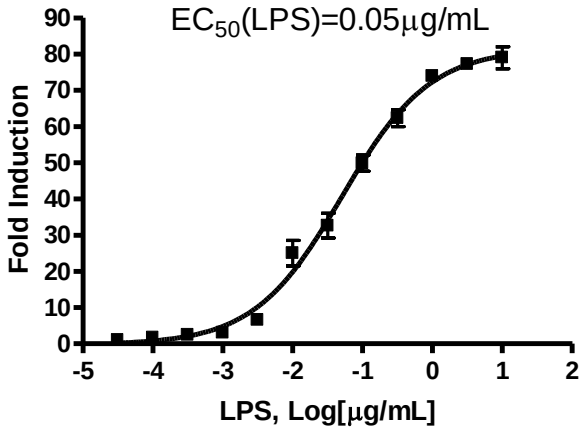

Figure 3. Generation of a stable NF-κB luciferase reporter THP-1 cell line using NF-κB luciferase reporter lentivirus. The NF-κB luciferase reporter THP-1 stable cell line was generated by transduction of THP-1 cells with NF-κB luciferase reporter lentivirus, followed by selection of a clonal cell line that has integrated the NF-κB luciferase reporter into the chromosome. Stimulation of the NF-κB reporter luciferase THP-1 stable cell line using LPS results in ~80-fold induction in NF-κB reporter activity.

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Related Products

Product	Cat. #	Size
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 rxns
NF-κB reporter (Luc) - HEK293 Cell line	60650	2 vials
NF-κB Reporter (Luc) - A549 Cell Line	60625	2 vials
NF-κB Reporter (Luc) - HCT116 Cell Line	60623	2 vials
NF-κB Reporter (Luc) - CHO-K1 Cell Line	60622	2 vials
NF-κB Reporter (Luc) - Jurkat Cell Line	60651	2 vials
NF-κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml

References

1. Pessara, U., Koch, N. (1990) Tumor necrosis factor alpha regulates expression of the major histocompatibility complex class II-associated invariant chain by binding of an NF-κB-like factor to a promoter element. *Mol Cell Biol*. **10(8):**4146-4154.
2. Baeuerle, P.A. (1998) Pro-inflammatory signaling: last pieces in the NF-κB puzzle? *Curr Biol*. **8(1):**R19-R22.