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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The NF-κB eGFP Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. The particles contain an enhanced GFP gene driven by 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 examining eGFP expression.

Application

- Screen for activators or inhibitors of NF-κB signaling pathway in transduced target cells
- Generation of NF-κB eGFP reporter stable cell line

Formulation

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

Titer

Two vials (500 µl x 2) of NF-κB eGFP 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 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.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

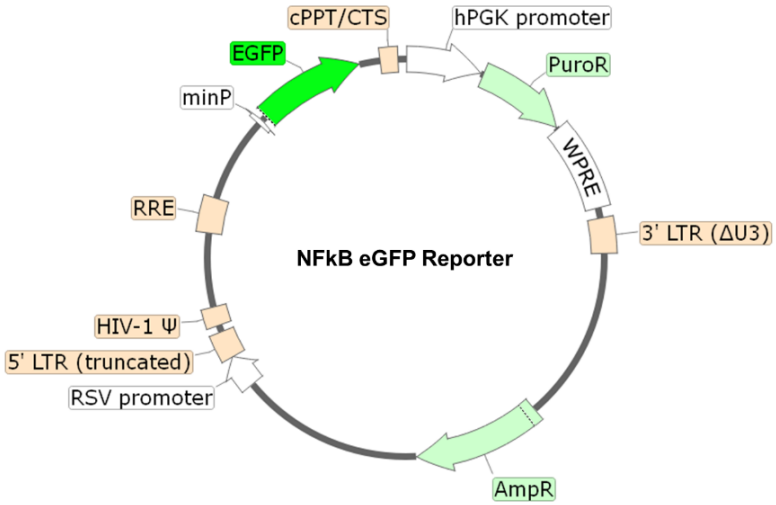

Figure 1. Schematic of the lenti-vector used to generate the NF-κB eGFP reporter lentivirus

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
TNFα	Sigma #T0157-10UG
Thaw Medium 9	BPS Bioscience #79665
Polybrene	Millipore #TR-1003-G
96-well white clear-bottom assay plate	Corning #3610
Negative Control eGFP Reporter Lentivirus	BPS Bioscience #79927
ONE-STEP Luciferase Assay System	BPS Bioscience #60690

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using NF-κB eGFP 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 requirements. 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. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of NF-κB eGFP 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 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. Day 4: prepare diluted TNFα in Thaw Medium 9. Add 10 µl of diluted TNFα to the TNFα-stimulated wells. Add 10 µl of Thaw Medium 9 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 24 hours.

6. The expression of eGFP can be analyzed by microscopy or flow cytometry (Ex/Em=488/510 nm)

Important Notes

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

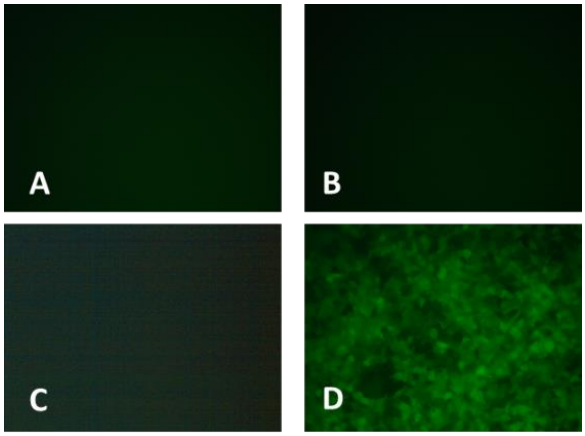

Figure 2. NF-κB eGFP reporter activity stimulated by TNFα in HEK293 cells. Approximately 10,000 HEK293 cells/well were infected with 100,000 TU/well negative control eGFP reporter (BPS Bioscience, #79927) or NF-κB eGFP reporter lentivirus. After 48 hours of transduction, medium was changed to HEK growth medium, and the cells were treated with 100 ng/ml TNFα for 24 hours. The expression of eGFP in the target cells was observed under a fluorescence microscope. A, negative control eGFP reporter without TNFα treatment; B, negative control eGFP reporter treated with TNFα; C, NF-κB eGFP reporter without TNFα treatment; D, NF-κB eGFP reporter treated with TNFα.

Related Products

Products	*Catalog #*	*Size*
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x 2
Negative control eGFP Reporter Lentivirus	79927	500 µl x 2
NFAT eGFP Reporter Lentivirus	79922	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (G418)	79980-G	500 µl x 2
NF-κB (GFP) – Reporter HEK293 Recombinant Cell Line	79402	2 vials
Thaw Medium 9	79665	100 ml