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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The NFκB Luciferase-eGFP Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and nondividing cells. The particles contain a firefly luciferase and eGFP (enhanced green fluorescent protein) cassette driven by the NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) response element located upstream of the minimal TATA promoter. These lentiviruses also transduce a puromycin selection marker (Figure 1). After transduction, activation of the NFκB signaling pathway in the target cells can be monitored by measuring luciferase activity or/and eGFP expression.

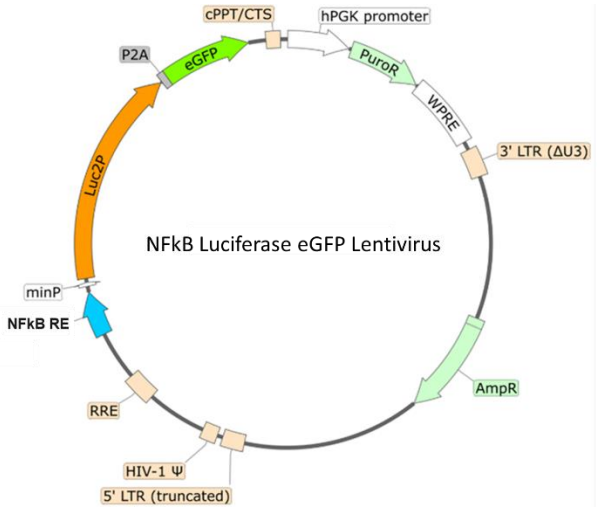

Figure 1. Schematic of the lenti-vector used to generate the NFκB Luciferase-eGFP Reporter Lentivirus.

Background

The role of NFκB (nuclear factor kappa-light chain enhancer of activated B cells) activation is well-characterized in canonical (classical) and noncanonical (alternative) signaling pathways of inflammation. Two major forms of innate immune sensors are Toll-like receptors (TLR) and NOD/CATERPILLER proteins. Mutations in NOD2 (nucleotide-binding oligomerization domain-containing protein 2) have been linked to chronic autoinflammatory and autoimmune diseases, such as Crohn's disease and Blaus syndrome. Studying the canonical and noncanonical NF-κB pathways and the influence of TLR pathways and NOD2 mutations can further our understanding of autoimmune regulation. The use of luciferase and eGFP (enhanced green fluorescence protein) reporter allows for easy read outs in cellular assays.

Application

- Screen for activators or inhibitors of NFκB signaling pathway in transduced target cells.
- Generate NFκB luciferase-eGFP reporter cell pools or stable cell lines following puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of NFκB Luciferase-eGFP Reporter Lentivirus at ≥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 lentiviruses at -80°C for up to 12 months from date of receipt. 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.

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
Tumor Necrosis Factor-α Human	Sigma #T0157-10UG
Thaw Medium 9	BPS Bioscience #79665
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well white clear-bottom assay plate	Corning #3610

Assay Protocol

- The following protocol is a general guideline for transducing HEK293 cells. 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with puromycin prior to carrying out the assays.
- The assay should include “Stimulated” and “Unstimulated” wells.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well in 50 µl of Thaw Medium 9 into white opaque 96-well microplate.
2. Add 5 µl of NFκB Luciferase-eGFP Reporter Lentivirus into each well.
3. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 µg/ml.
4. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2.

Day 3:

1. Prepare diluted human TNFα in Thaw Medium 9 at the desired final concentration (100 µl/well).

2. Remove the medium containing the lentivirus from the wells.

3. Add 100 µl of diluted human TNFα to the "Stimulated" wells.

4. Add 100 µl of Thaw Medium 9 to the "Unstimulated" control wells (for measuring the uninduced level of NFκB Luciferase-eGFP reporter activity).

5. Incubate at 37°C with 5% CO_2 for 24 hours.

Day 4:

1. The expression of GFP can be analyzed by microscopy or flow cytometry, or another method of interest.

2. Prepare the ONE-Step™ Luciferase as recommended in the product protocol (#60690) (100 µl/well). Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

3. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes

To generate an NFκB luciferase-eGFP reporter cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, https://bpsbioscience.com/kill-curve-protocol) for antibiotic selection of transduced cells, followed by clonal selection.

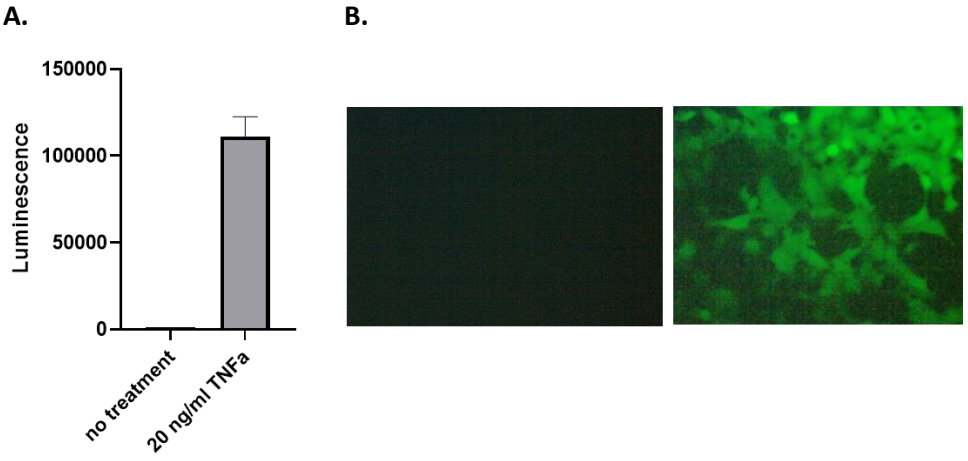

Figure 2. NFκB reporter activity (luciferase and eGFP expression) in HEK293 cells stimulated by human TNFα and transduced with NFκB Luciferase-eGFP Reporter Lentivirus.
Approximately 8,000 cells/well of HEK293 cells were transduced with 100,000 TU/well of NFκB Luciferase-eGFP Reporter Lentivirus. 48 hours post-transduction, cells were stimulated with 20 ng/ml of human TNFα for 24 hours. **A**. Luciferase activity results are shown as the raw luminescence readings. **B.** eGFP expression was observed under a fluorescence microscope (left panel: unstimulated cells; right panel: cells stimulated with 20 ng/ml of human TNFα).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

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

References

Pessara U., Koch N., 1990 *Mol Cell Biol.* 10(8):4146-4154.
Baeuerle P.A., 1998 *Curr Biol.* 8(1): R19-R22.
Treisman R., 1992 *Trends Biochem Sci.* 17(10): 423-426.

Related Products

Products	*Catalog #*	*Size*
NFκB Luciferase Reporter Lentivirus	79564	500 µl x 2
NFκB eGFP Reporter Lentivirus	79926	500 µl x 2

Version 081524