Description

The NF-κB SEAP 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 SEAP (Secreted Embryonic Alkaline Phosphatase) reporter driven by the NF-κB (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 NF-κB signaling pathway in the target cells can be monitored by measuring SEAP activity.

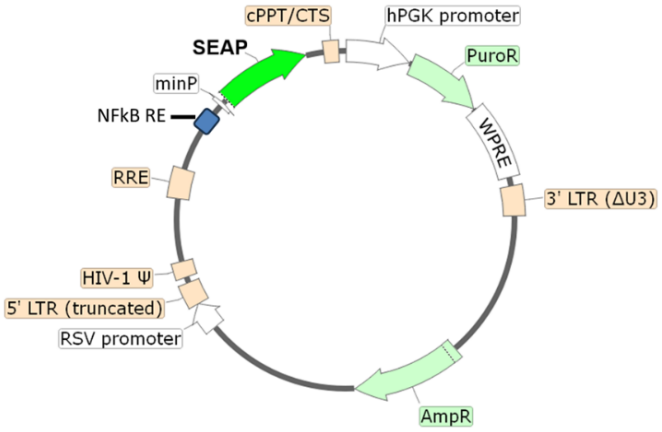

Figure 1. Schematic of the lenti-vector used to generate the NF-κB SEAP Reporter Lentivirus.

Background

The role of NF-κB (nuclear factor kappa-light chain enhancer of activated B cells) 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 reporter systems allows for easy assay read outs. SEAP (Secreted Embryonic Alkaline Phosphatase) is a reporter protein that has been extensively used to assess the activity of transcription factors of interest. Since SEAP is secreted, cell lysis is not required to measure reporter activity, simplifying the assay.

Application

- Screen for activators or inhibitors of NF-κB signaling pathway in transduced target cells.
- Generate N-FκB SEAP 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 NF-κB SEAP 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 21	BPS Bioscience #84211
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Quanti Blue™	Invivogen # rep-qbs2
NovaBright™ Phospha-Light™ EXP Assay Kit	Thermofisher #N10577
96-well transparent cell culture plate (for colorimetry) OR	Genesee Scientific #25-109
96-well white clear-bottom assay plate (for chemiluminescence)	Corning #3610
Spectrophotometer OR Luminometer	

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 Control” wells.

Day 1:

1. Seed HEK293 cells at a density of 5,000-10,000 cells per well in 100 µl of Thaw Medium 21 into a white opaque 96-well cell culture plate.
2. Add 5 µl of NF-κB SEAP 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 21 at the desired final concentration (100 µl/well).
2. Remove the medium containing the lentiviruses from the wells.
3. Add 100 µl of diluted human TNFα to the "Stimulated" wells.
4. Add 100 µl of Thaw Medium 21 to the "Unstimulated Control" wells (for measuring the uninduced level of NF-κB SEAP reporter activity).
5. Incubate at 37°C with 5% CO_2 for 24 hours.

Day 4:

1. Transfer 20 µl of supernatant from each well to a new plate for SEAP expression measurement. SEAP expression can be measured using colorimetry-based detection (use a transparent 96-well plate) or chemiluminescence-based detection (use a 96-well tissue culture-treated white clear-bottom plate), following manufacturer's instructions.

Important Notes

To generate an NF-κB SEAP 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.

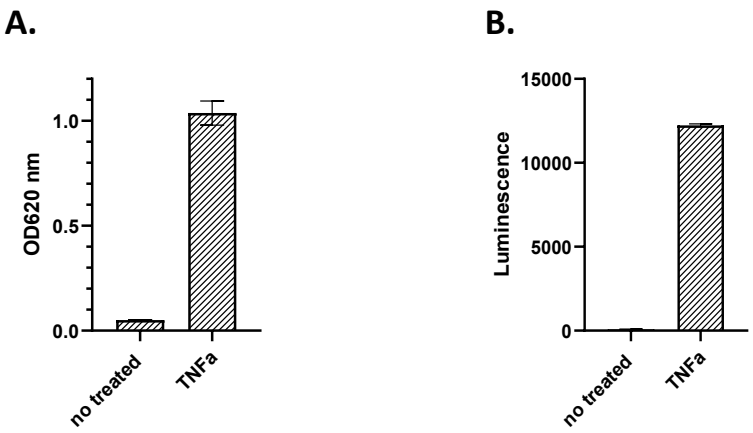

Figure 2. SEAP reporter activity in HEK293 cells transduced with NF-κB SEAP Reporter Lentivirus stimulated by human TNFa.

Approximately 8,000 HEK293 cells/well were transduced with 100,000 TU/well of NF-κB SEAP Reporter Lentivirus. 48 hours post-transduction, cells were stimulated with 10 ng/ml of human TNFα for 24 hours and SEAP activity was measured. **A**. SEAP activity results are shown as raw OD620nm, as measured using Quanti Blue™ (Invivogen # rep-qbs2). **B.** SEAP activity results are shown as raw luminescence, as measured using NovaBright™ Phospha-Light™ EXP Assay Kit (Thermofisher #N10577).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Pessara U. and 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.

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