Description

NF-κB mCherry 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 non-dividing cells. The particles contain an mCherry reporter under the control of the NF-κB (nuclear factor kappa-light chain enhancer of activated B cells) response element located upstream of the minimal TATA promoter. The lentiviruses also transduce a puromycin selection gene (Figure 1). After transduction, activation of the NF-κB signaling pathway in target cells can be monitored by assessing mCherry expression.

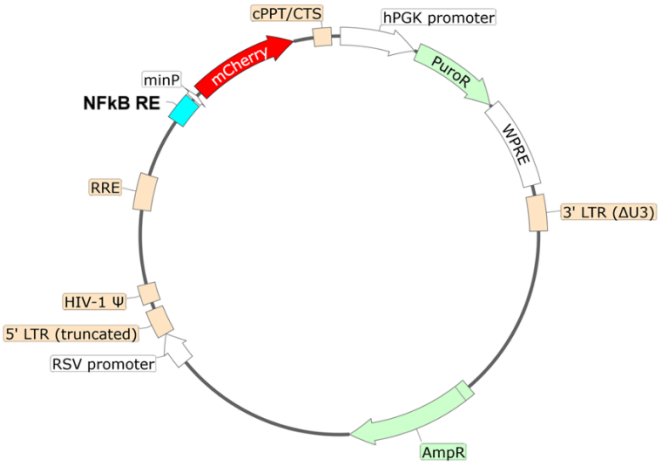

Figure 1. Schematic of the lenti-vector used to generate the NF-κB mCherry Reporter Lentivirus.

Background

The role of NF-κB (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.

mCherry is a monomeric red fluorescent protein derived from DsRed found in the sea anemones *Discosoma*. It belongs to the mFruit family of monomeric red fluorescent proteins, which are improved versions of mRFP1 (monomeric red fluorescent protein 1) in terms of brightness and photostability. The use of fluorescent proteins allows for direct visualization of stimulated cells under a fluorescent microscope or analysis by flow cytometry.

Application

- Screen for activators or inhibitors of the NF-κB signaling pathway in transduced target cells.
- Generate NF-κB mCherry reporter cell pools or stable cell lines following puromycin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer >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 the date of receipt. Avoid repeated freeze/thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

Biosafety

The lentiviruses are produced with 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience 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 protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
293 [HEK-293]	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Human TNFα	Sigma #T0157-10UG
96-well tissue culture, clear-bottom, white plate	Corning #3610

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for Cell Culture and Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Protocol

- The following protocol is a general guideline for transducing HEK293 cells using the NF-κB mCherry 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 can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells expressing the reporter with puromycin, creating a cell pool or stable cell line, prior to carrying out the reporter assays.
- The assay should include "Stimulated", "Cell-Free Control" and "Control Untreated" conditions.

Day 1:

1. Seed HEK293 cells at a density of ~8,000 cells per well in 90 µl of Thaw Medium 1 into a white, clear bottom 96-well microplate.

2. To each well, add 5 µl of NF-κB mCherry Reporter Lentivirus.

   Optional: Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer (#78939) to each well to a final concentration of 5 µg/ml.

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 for 48 hours.

Day 3:

1. 48 hours after transduction, remove the medium containing the lentiviruses from each well.

2. Add 100 µl of Thaw Medium 1 containing the compounds being tested to the "Stimulated" wells.

3. Add 100 µl of Thaw Medium 1 to the "Control Untreated" wells (to determine the unstimulated mCherry fluorescence from the transduced HEK293 cells).

4. Add 100 µl of Thaw Medium 1 to the "Cell-Free Control" wells (to determine the background fluorescence).

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

Day 4:

1. The expression of mCherry can be analyzed by a fluorescence microscope or flow cytometry, or another method of interest.

Important Notes

To generate an NFκB mCherry reporter stable 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, Kill Curve Protocol) for antibiotic selection of transduced cells, followed by clonal selection.

Validation Data

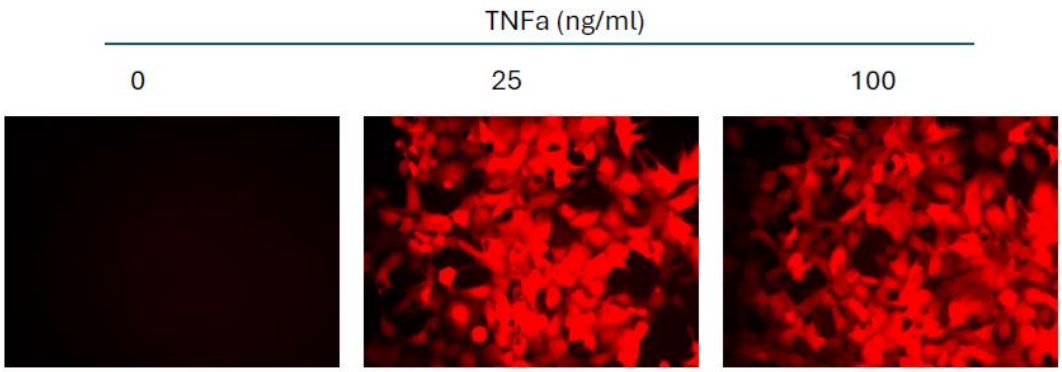

Figure 2. Activation of mCherry reporter activity in HEK293 cells transduced with NF-κB mCherry Reporter Lentivirus.
Approximately 8,000 HEK293 cells/well were transduced with 100,000 TU/well of NF-κB mCherry Reporter Lentivirus. 48 hours post-transduction, cells were stimulated with 25 ng/ml or 100 ng/ml of human TNFα for 24 hours. The expression of mCherry was observed under a fluorescence microscope.

Data shown is representative.

References

Pessara U. and Koch N., 1990 *Mol Cell Biol*. 10(8):4146-4154.
Baeuerle P.A., 1998 *Curr Biol.* 8(1):R19-R22

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.

Related Products

Products	*Catalog #*	*Size*
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
NFkB eGFP Reporter Lentivirus	79926	500 µl x 2
NFkB Luciferase Reporter Lentivirus	79564	500 µl x 2

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