Description

NLR family Pyrin domain containing 3 (NLRP3) is expressed in macrophages and is a component of inflammasomes. NLRP3 detects uric acid and extracellular ATP in damaged tissue and interacts with a pro-apoptotic protein that recruits caspases. This complex is also an upstream activator of NF-κB signaling and triggers an immune response as part of the innate immune system. Mutations in NLRP3 are known to cause autoinflammatory and neuroinflammatory diseases such as Alzheimer's, Parkinson's, and prion disease.

The NLRP3 CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based VSV-G pseudo-typed lentiviral particles ready to be transduced into most types of mammalian cells, including primary and non-dividing cells. The particles contain a CRISPR/Cas9 gene driven by an EF1A promoter, along with 5 sgRNA (single guide RNA) targeting human NLRP3 (Figure 1 and Table 1), allowing the knockdown of NLRP3 in transduced cells

The non-integrating lentivirus is made with a mutated integrase, resulting in only transient expression of the Cas9 and sgRNA. Although using the non-integrating lentivirus results in lower knockdown efficiency, the Cas9 protein is not permanently expressed, which lowers the risk of off-targeting, and there are no random integrations into the cell's genome Despite transient expression of Cas9 and sgRNA, knockout cell lines can be generated using cell sorting or limiting dilution due to the permanent changes in the genomic DNA from the Cas9 nuclease activity and NHEJ repair.

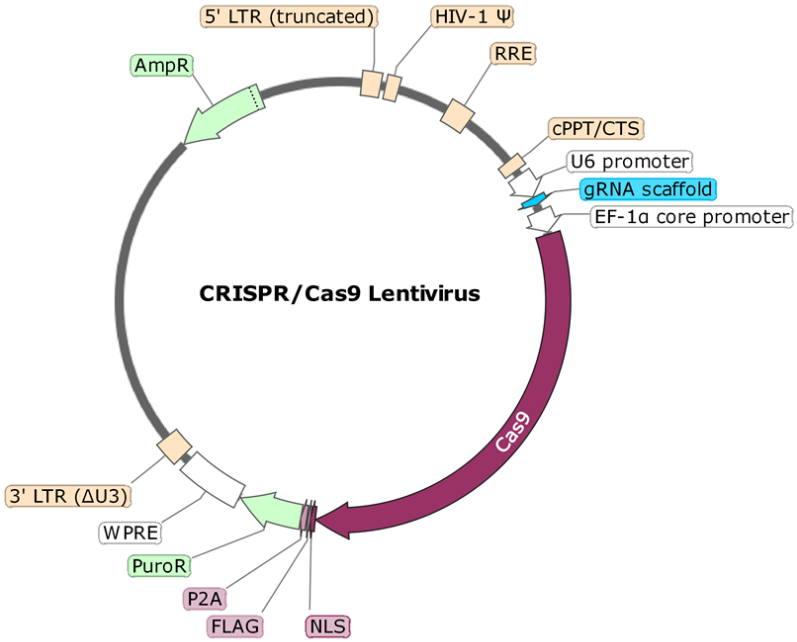

Figure 1: Schematic of the lenti-vector used to generate the NLRP3 CRISPR/Cas9 Lentivirus.

Gene Target:	sgRNA Sequence:
NLRP3	GGTGCCTTTGACGAGCACAT
NLRP3	AAAAGAGATGAGCCGAAGTG
NLRP3	GTTCTATATCCACTGTCGAG
NLRP3	AGAGATTGATCTCAATCTTG
NLRP3	GAAGACAGGAATGCCCGTCT

Table 1: List of sgRNA Sequences in the NLRP3 CRISPR/Cas9 Lentivirus.

Application(s)

- Transient knockdown of NLRP3 in target cells
- Generation of a stable NLRP3 knockout cell line following transient puromycin selection and limiting dilution cloning

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of 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 lentiviruses 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. 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.

Validation Data

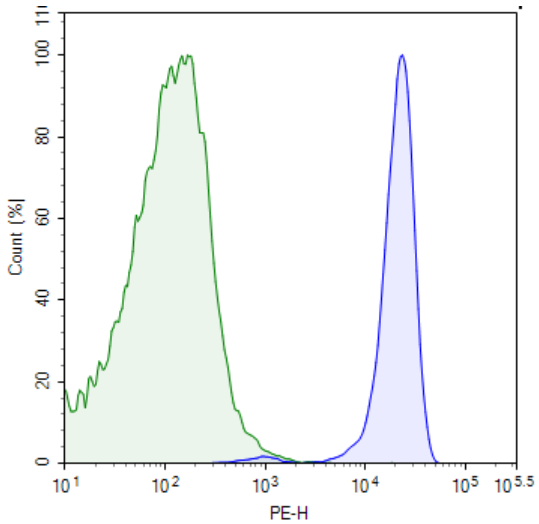

Figure 2: Knockdown of NLRP3 in THP-1 cells using NLRP3 CRISPR/Cas9 Lentivirus.
THP-1 cells were transduced via spinoculation with 8 x 10^7 TU/well of NLRP3 CRISPR/Cas9 lentivirus, corresponding to an MOI of approximately 5-10. 48 hours after transduction, cells were enriched using transient 0.5 µg/ml puromycin for 48 hours, stained with PE-labeled anti-human NLRP3 polyclonal antibody (Invitrogen #79740), and analyzed by flow cytometry. Non-transduced, parental THP-1 cells are shown in blue, and the transduced cells are shown in green.

Troubleshooting Guide

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

License Disclosure

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

Related Products

Products	*Catalog #*	*Size*
CTLA4 CRISPR/Cas9 Lentivirus (Non-Integrating)	78061	500 µl x 2
CTLA4 CRISPR/Cas9 Lentivirus (Integrating)	78054	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Integrating)	78058	500 µl x 2
CD47 CRISPR/Cas9 Lentivirus (Non-Integrating)	78063	500 µl x 2
CD47 CRISPR/Cas9 Lentivirus (Integrating)	78056	500 µl x 2
FCGR2A CRISPR/Cas9 Lentivirus (Non-Integrating)	78538	500 µl x 2
FCGR2A CRISPR/Cas9 Lentivirus (Integrating)	78537	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Non-Integrating)	78536	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Integrating)	78535	500 µl x 2