Description

The NLRP3 shRNA Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These particles contain 3 shRNAs (Short hairpin RNA) targeting human NLRP3 driven by a U6 promoter, and a puromycin selection marker (Figures 1). The sequences of the shRNA used are shown in Table 1.

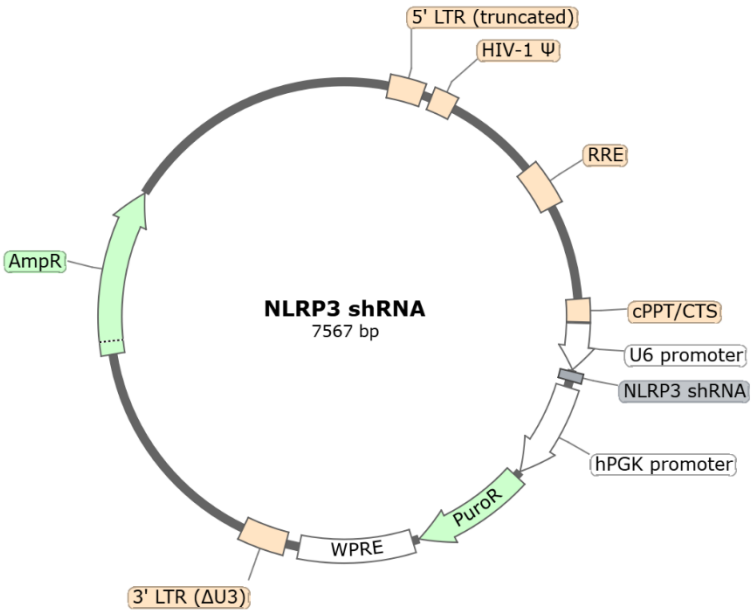

Figure 1. Schematic of the lenti-vector used to generate the NLRP3 Human shRNA Lentivirus.

Table 1: List of shRNA sequences present in the NLRP3 shRNA Lentivirus.

Gene Target:	shRNA Sequence
NLRP3	GAGACTCAGGAGTCGCAATTT
NLRP3	GGCTGTAACATTCGGAGATTG
NLRP3	TCATCATTCCCGCTATCTTTC

Background

NOD, LRR and pyrin domain containing 3 (NLRP3), also known as NALP3 and cryopyrin, is a pattern recognition receptor (PRR) of the NRL (NOD-like receptor) subfamily. It is involved in the detection of microbes, endogenous and exogenous stress signals. It is expressed in macrophages and when bound to PYCARD (adaptor ASC protein) forms a caspase-1 activating complex named NRLP3 inflammasome. NLRP3 detects uric acid and extracellular ATP in damaged cells, and once activated it leads to an immune response. Upon activation, NLRP3 inflammasome releases its partners HSP90 and SGT1, and binds to PYCARD and caspase-1. Caspase-1 initiates the processing and release of the pro-inflammatory cytokines IL-1β and IL-18 and gasdermin D-mediated pyroptotic cell death. Mutations in NLRP3 are known to cause autoinflammatory and neuroinflammatory diseases, such as Alzheimer's, Parkinson's, and prion disease. NLRP3 is the most extensively studied inflammasome protein to date due to its array of activators and aberrant activation in several inflammatory diseases. Studies into its function and inhibition can lead to the development of therapeutic avenues for the treatment of auto-inflammatory diseases.

Application

- Generate a NLRP3 knockdown cell pool following puromycin selection.
- Generate a NLRP3 knockdown cell line following puromycin selection and limiting dilution.

Formulation

The lentivirus particles were produced from HEK293T cells in 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 ≥5 x 10^6 TU/ml. The titer varies 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 a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after 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.

Notes

To generate a NLRP3 knockdown 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, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, following by clonal selection.

Figures and Validation Data

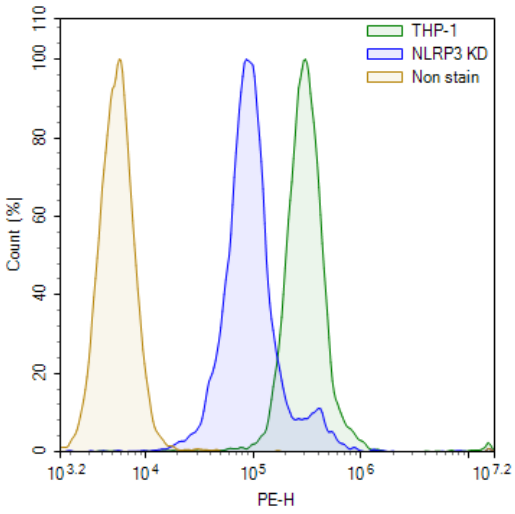

Figure 2. NLRP3 expression levels in THP-1 cells after transduction with NRLP3 Human shRNA Lentiviruses.
Parental THP-1 cells were transduced by spinoculation with NLRP3 shRNA lentivirus. 24 hours after transduction, transduced cells were selected with puromycin for another 24 hours and stained with anti- human NLRP3 polyclonal antibody (Invitrogen #PA5-79740) and analyzed by flow cytometry. Parental THP-1 cells are shown in green, and the transduced cells are shown in blue. Non-stained cells were used as negative control (yellow). The Y-axis represents the % cell number. The X-axis indicates the intensity of PE signal.

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

References

Swanson K *et al.*, 2019 *Nature Reviews Immunology* 19:477-489.

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
NLRP3 CRISPR/Cas9 Lentivirus (Integrating)	78545	500 µl x 2
NLRP3 CRISPR/Cas9 Lentivirus (Non-Integrating)	78546	500 µl x 2
NLRP3 (NALP3), His-FLAG-Tags (Sf9-derived) Recombinant	40741	10 µg
NLRP3 Knockdown THP-1 Cell Pool	82121	2 vials