

Description

Cas9 (*Streptococcus pyogenes* CRISPR associated protein 9) is an endonuclease enzyme that, when recruited to a specific DNA sequence by the sgRNA (single guide RNA), introduces a double stranded break into the DNA. This double stranded break is repaired in mammalian cells either through Non-Homologous End Joining or Homologous Recombination. Non-Homologous End Joining often results in the deletion or insertion of several base pairs at the cut site, which, when resulting in a frameshift, causes the functional inactivation of the targeted gene.

Cas9 Lentivirus can be used to generate Cas9 expressing cells in almost any mammalian cell line. Cells stably expressing Cas9 can then be transduced or electroporated with sgRNA targeting a gene of interest to quickly generate knock-out cell pools or cell lines.

The Cas9 Lentiviruses are replication incompetent, HIV-based VSV-G pseudo-typed lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a Cas9 gene driven by an EF1A promoter, along with a Neomycin selection marker.

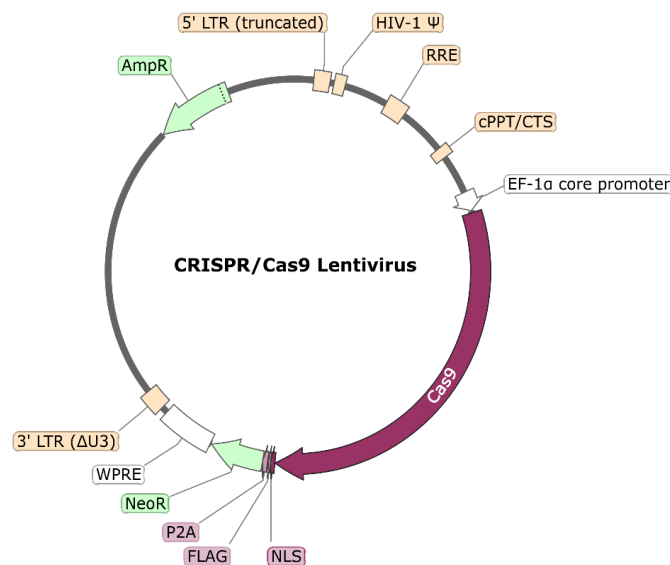


Figure 1: Schematic of the lenti-vector used to generate the Cas9 (neomycin selection) lentivirus.

Application(s)

- Transient expression of Cas9 in target cells
- Generation of a stable Cas9 over-expressing cell line following neomycin selection

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 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^7$ 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 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.

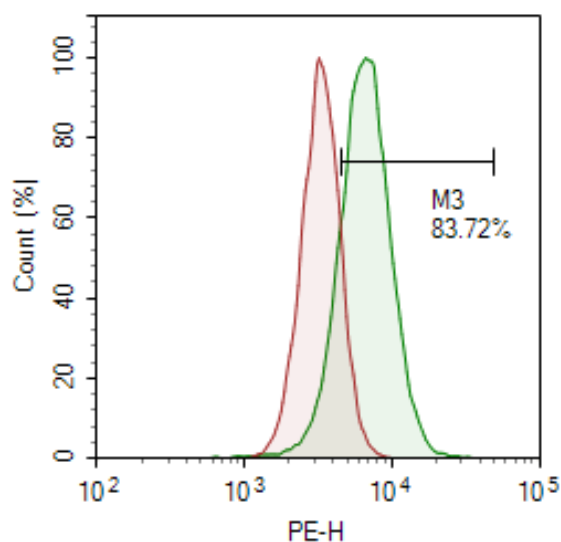
Figures and Validation Data

Figure 2: Expression of Cas9 in Jurkat Cells. Jurkat cells were transduced via spinoculation with Cas9 Lentivirus (Neomycin selection) at an MOI of 5-10. 48 hours after transduction, cells were stained with PE-labeled anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental Jurkat cells are shown in red, 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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Cas9 Lentivirus (Puromycin)	78066	500 µl x 2
Cas9, His-tag (<i>S. pyogenes</i>)	100206-1	50 µg
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Integrating)	78058	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
Cas9 Expressing Jurkat Cell Pool	78070	2 vials
Cas9 Expressing MDA-MB-231 Cell Pool	78069	2 vials
Cas9 Expressing A549 Cell Pool	78072	2 vials
Cas9 Expressing HCT116 Cell Pool	78073	2 vials
Cas9 Expressing Raji Cell Pool	78071	2 vials