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Data Sheet

Cas9 Lentivirus (Puromycin Selection)

Catalog #: 78066

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 Puromycin selection marker.

Application

1. Transient expression of Cas9 in target cells.
2. Generation of a stable Cas9 over-expressing cell line following puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^6$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

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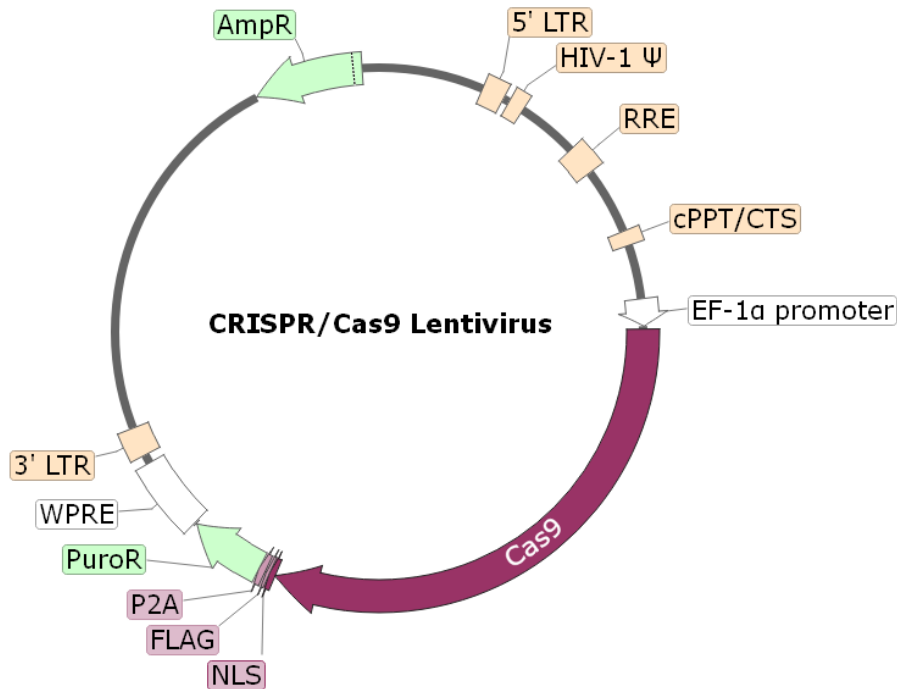


Figure 1. Schematic of the Lenti-vector used to generate the Cas9 Lentivirus.

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

None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells. Although the pseudotyped lentiviruses are replication-incompetent, they do require the use of a Biosafety Level 2 facility. BPS recommends following all federal, state, local, and institutional regulations and using all appropriate safety precautions.

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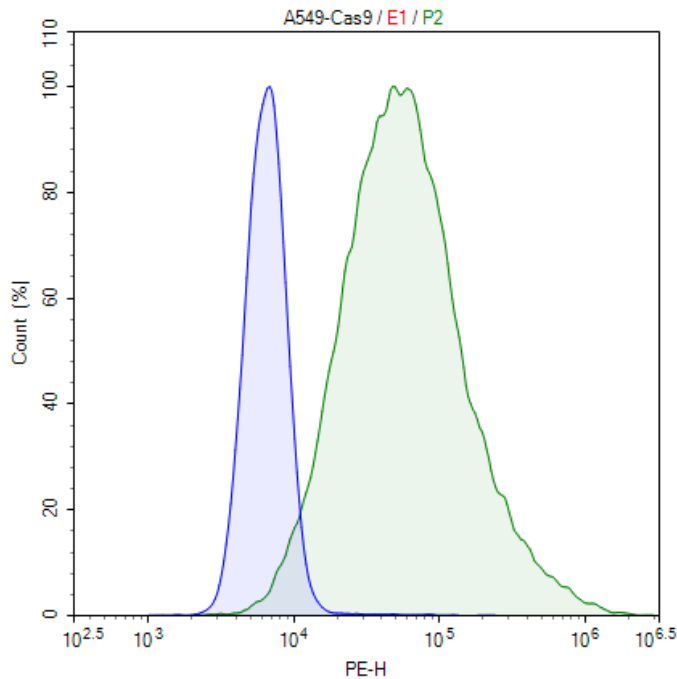


Figure 2. Transduction of Cas9 Lentivirus in A549 cells.

A549 cells were transduced with 5,000,000 TU/well of Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-FLAG antibody (BioLegend, #637309) and analyzed by FACS. Parental A549 cells are shown in blue, and the transduced cells are shown in green.

Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Cas9, His-tag (<i>S. pyogenes</i>)	100206-1	50 µg
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 cells	78070	2 vials
Cas9 Expressing MDA-MB-231 cells	78069	2 vials
Cas9 Expressing A549 cells	78072	2 vials
Cas9 Expressing HCT116 cells	78073	2 vials
Cas9 Expressing Raji cells	78071	2 vials

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Notes

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.

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