Description

Cas9 Lentiviruses (Inducible Tet-On) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses transduce cells with the *Streptococcus pyogenes* Cas9 gene under a tight TRE tetracycline-inducible promoter. Cas9 expression in the transduced cells is induced with doxycycline treatment, allowing temporal control of its expression, and when combined with sgRNA targeting gene(s) of interest allows gene editing events to be temporally controlled. The lentivirus vector also contains a geneticin selection gene (Figure 1).

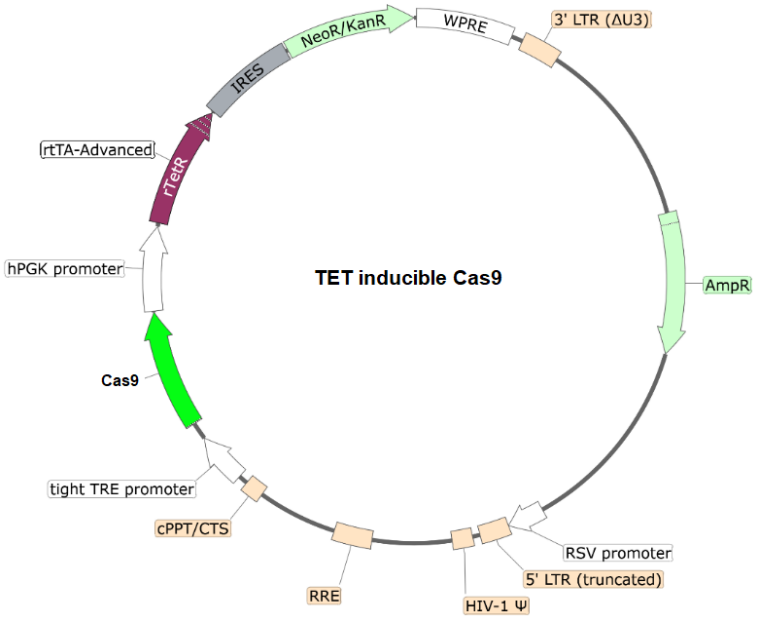


Figure 1. Schematic of the lenti-vector used to generate the Cas9 lentivirus (Inducible Tet-On).

Background

Cas9 (*Streptococcus pyogenes* CRISPR associated protein 9) is an endonuclease that, when recruited to a specific DNA sequence by the appropriate sgRNA (single guide RNA), introduces a double stranded break into the DNA. This double stranded break can then be repaired through either Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR). While NHEJ is an error prone process and causes insertions or deletions which may result in functional inactivation of the target gene, HR, in conjunction with a single stranded ssDNA repair construct, can be used to introduce mutations at specific base pair(s). Gene modifications introduced via Cas9 are now used in multiple fields of research aimed at understanding cellular mechanisms and developing therapeutic solutions.

Application(s)

Generation of Tet-inducible Cas9 stable cell lines (Geneticin (G418) selection) for genetic engineering studies.

Formulation

The lentiviruses 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 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 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.

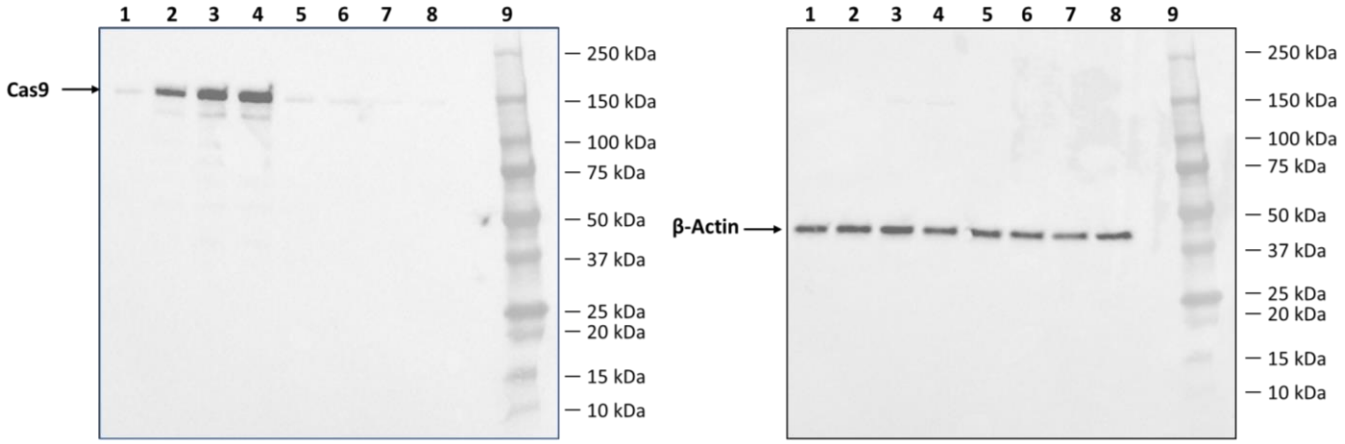
Note

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.

Troubleshooting Guide

For all questions, please email support@bpsbioscience.com

Validation Data



Lane 1: Untreated Cas9 Inducible (Tet-On) iPS Cell Pool

Lane 2: Cas9 Inducible (Tet-On) iPS Cell Pool + 0.5 µg/mL Doxycycline

Lane 3: Cas9 Inducible (Tet-On) iPS Cell Pool +1 µg/mL Doxycycline

Lane 4: Cas9 Inducible (Tet-On) iPS Cell Pool +1.5 µg/mL Doxycycline

Lane 5: Untreated Cas9 Inducible (Tet-On) iPS Cell Pool

Lane 6: 96 hours post 0.5 µg/mL Doxycycline

Lane 7: 96 hours post 1 µg/mL Doxycycline

Lane 8:96 hours post 1.5 µg/mL Doxycycline

Lane 9: Protein Marker

Figure 2. Cas9 protein expression in Cas9-Expressing iPS Cell Pool assessment by western blot. The Cas9 Inducible (Tet-On) iPS Cell Pool (BPS Bioscience #78845) was generated by transduction of iPSC with Cas9 Lentivirus (Inducible Tet-On), followed by geneticin (G418) selection. Cas9 Inducible (Tet-On) iPS Cells were collected and analyzed by western blot after 72 hours of treatment with various concentrations of doxycycline or 96 hours after withdrawal of doxycycline media. Cell lysate was analyzed by western blot using either Mouse Anti Cas9 (Biolegend #844301) or Rabbit Anti Actin (Cell Signaling Technology #4970) primary antibody and Anti-Mouse (Biolegend #405306) or Anti-Rabbit (SBCT #2357) HRP conjugated secondary antibodies respectively.

Related Products

Products	*Catalog #*	*Size*
Cas9 Lentivirus (Puromycin)	78066	500 µl x 2
Cas9, His-tag (S. pyogenes)	100206-1	50 µg
Cas9 Expressing iPS Cell Pool	78578	1 vial
Cas9 Inducible (Tet-On) iPS Cell Pool	78845	1 vial
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