Description

Cas9 is an endonuclease enzyme that introduces a double stranded break into the DNA when recruited to a specific DNA sequence by the sgRNA (single guide RNA). 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 gene.

SaCas9 (*Staphylococcus aureus* CRISPR-associated protein 9) has demonstrated high cutting efficiency in mammalian cells, and its smaller size makes it ideal for packaging into AAV. SaCas9 recognizes a longer protospacer adjacent motif (PAM) site, 5'-NNGRRT-3', than the more traditional SpCas9 (*Streptococcus pyogenes* CRISPR-associated protein 9). These AAV particles constitutively express SaCas9 under the control of a CMV promoter.

Adeno-Associated Virus-DJ (AAV-DJ) is a synthetic serotype made from eight different wild-type AAV serotypes (AAV2, 4, 5, 8, 9, avian, bovine, and goat AAV) using DNA shuffling. These modifications give the AAV-DJ serotype improved transduction efficiency in vitro and in vivo compared to wild-type serotypes. Consequently, AAV-DJ can infect a broad range of cell types.

Application(s)

Generate a SaCas9 over-expressing cell line for knockout or knock-in studies

Serotype

AAV-DJ

Formulation

AAV-DJ was produced in HEK293-AAV cells and is supplied in PBS-MK (PBS Magnesium-Potassium) buffer containing 0.01% Pluronic F68.

Purification

The purity of the AAV particles was confirmed to be greater than 90% by staining with One-Step Lumitein™ UV Protein Gel Stain (Biotium #21005-1L). The purity will vary with each lot; the exact value will be provided with each shipment.

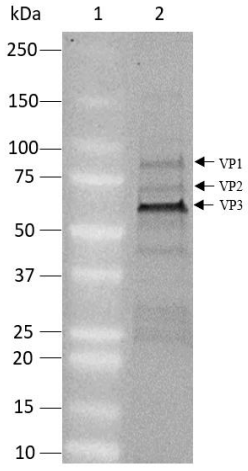

Figure 1. Purified AAV-DJ SaCas9 particles.
Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and 2 x 10^9 VG (vector genome) of AAV-DJ SaCas9 is in lane 2. AAV viral proteins VP1, VP2, and VP3 are labeled.

Titer

Two vials (50 µl x 2) of AAV at a titer ≥1 x 10^{12} vector genomes/ml. The titer is determined by qPCR and will vary with each lot; the exact value will be provided with each shipment.

Storage

AAV is shipped with dry ice. For long-term storage, it is recommended to store AAV at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

Recombinant AAV is inherently replication-deficient and not known to cause any human diseases. Additionally, following transduction, AAV vectors exist episomally and do not integrate into or disrupt the host cell's genome. AAV requires the use of a Biosafety Level 1 facility. BPS Bioscience recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

Validation Data

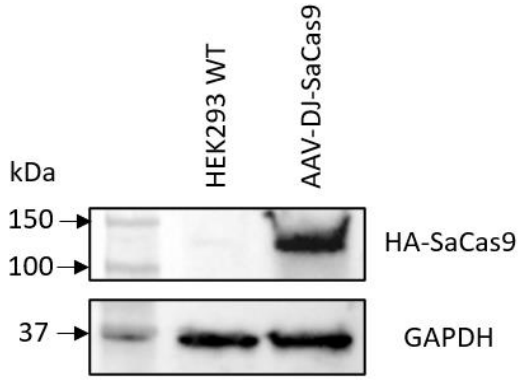

Figure 2. Transduction of HEK293 cells using AAV-DJ SaCas9.
1 x 10^5 cells/well were transduced in a 6-well plate with AAV-DJ SaCas9 at an MOI of 1 x 10^4. After 72 hours of transduction, SaCas9 expression in the target cells was detected by Western blot with an Anti-HA.11 Epitope Tag Antibody (BioLegend #901516).

Notes

The AAV-DJ viruses are covered under several patents, including U.S. Patent Nos. 7,588,772, 8,067,014, 8,574,583, and 8,906,387, as well as corresponding foreign patents applications and patent rights. AAV-DJ is used under a license agreement.

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*
AAV-DJ Luciferase-mCherry	78469	50 µl x 2
AAV-DJ ZsGreen	78442	50 µl x 2
AAV1 ZsGreen	78443	50 µl x 2
AAV3 Luciferase-eGFP	78463	50 µl x 2