Description
Adeno-Associated Virus Serotype 3 (AAV3) shares 82% sequence homology with AAV2, and like AAV2, requires the Heparan Sulfate Proteoglycan (HSPG) receptor for cell attachment. AAV3 vectors transduce human liver cancer cells extremely efficiently because AAV3 utilizes the human Hepatocyte Growth Factor Receptor (hHGFR) as a co-receptor for viral entry, which is highly expressed in these cells. Both the extracellular and intracellular kinase domains of hHGFR are required for AAV3-mediated transgene expression.

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.

Application(s)
Express SaCas9 to enable genome editing

Serotype
Wild-type AAV Serotype 3

Formulation
AAV 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.

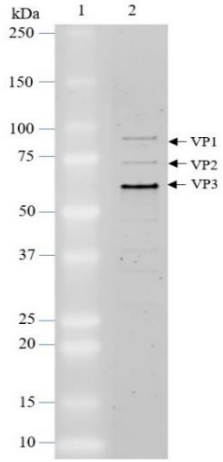

Figure 1. Purified AAV3 SaCas9 particles.
Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and 5 x 10^9 VG (vector genome) of AAV3 is shown in lane 2. AAV viral proteins VP1, VP2, and VP3 are labelled.

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

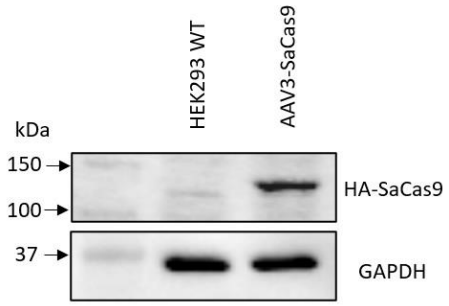

Figure 2. Transduction of HEK293 cells using AAV3 SaCas9.
1 x 10^5 cells were transduced in a 24-well plate with AAV3 SaCas9 at an MOI of 1 x 10^4. After 72 hours of transduction, SaCas9 expression in the target cells was measured by Western blot (Anti-HA.11 Epitope Tag Antibody, Biolegend #901516).

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*
AAV3 ZsGreen	78445	50 µl x 2
AAV9 ZsGreen	78450	50 µl x 2
AAV3 Luciferase-eGFP	78463	50 µl x 2
AAV9 Luciferase-eGFP	78468	50 µl x 2
AAV3 Luciferase-mCherry	78472	50 µl x 2
AAV5 Luciferase-mCherry	78474	50 µl x 2
AAV8 Luciferase-mCherry	78476	50 µl x 2
AAV1 SaCas9	78479	50 µl x 2
AAV2 SaCas9	78480	50 µl x 2