Description
Adeno-Associated Virus serotype 6 (AAV6) appears to be related to AAV1 by sequence analysis and shows the best transduction efficiency in pancreatic beta-cells compared to other AAV serotypes. AAV6 vectors are also particularly effective in the transduction of human prostate, breast, and liver cancer cells.

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 6

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.

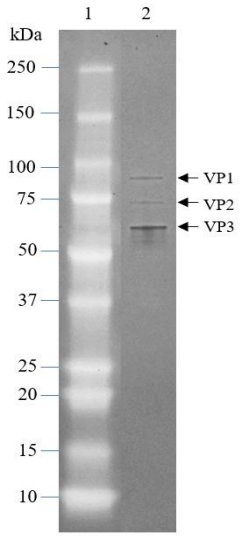

Figure 1. Purified AAV6 SaCas9 particles.
Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and 5 x 10^9 VG (vector genome) of AAV6 is shown in lane 2. AAV viral proteins VP1, VP2, and VP3 are labelled.

Titer

Two vials (50 µl x 2) of AAV at a titer ≥1 x 10^{11} 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

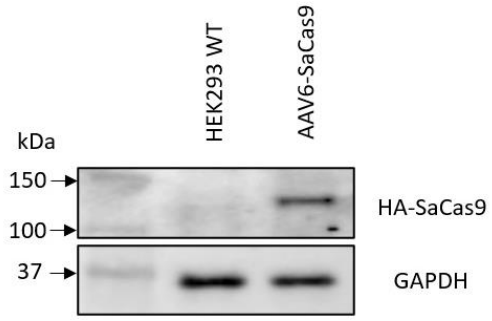

Figure 2. Transduction of HEK293 cells using AAV6 SaCas9.
1 x 10^5 cells were transduced in a 24-well plate with AAV6 SaCas9 at an MOI of 1 x 10^4. After 72 hours of transduction, SaCas9 expression in the target cells was measured by Western blot usingAnti-HA.11 Epitope Tag Antibody (Biolegend #901516).

Reference

Sayroo, R., *et al*. 2016. *Gene Ther*. **23:** 18–25. https://doi.org/10.1038/gt.2015.89

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
AAV6 ZsGreen	78448	50 µl x 2
AAV1 Luciferase	78452	50 µl x 2
AAV6 Luciferase	78457	50 µl x 2
AAV6 Luciferase-eGFP	78466	50 µl x 2
AAV6 Luciferase-mCherry	78475	50 µl x 2
AAV9 Luciferase-mCherry	78477	50 µl x 2
AAV2 SaCas9	78480	50 µl x 2