Description
Adeno-Associated Virus serotype 2 (AAV2) is the best characterized AAV serotype. Nearly all recombinant AAV serotypes utilize the AAV2 inverted terminal repeats (ITRs). AAV2 requires the expression of Heparan Sulfate Proteoglycan (HSPG) on the surface of host for cells for binding and internalization. Of nearly all the discovered AAV serotypes, AAV2 has the best transduction efficiency in cell culture and is the best tool for in vitro studies.

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. The protein contains a C-terminus HA tag.

Application(s)

- Express SaCas9 to enable genome editing

Serotype
Wild-type AAV Serotype 2

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). Purity will vary with each lot; the exact value will be provided with each shipment.

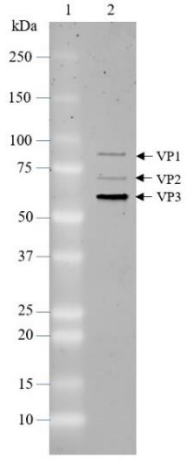

Figure 1. Purified AAV2 SaCas9 particles.
Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and 2 x 10^9 VG (vector genome) of AAV is shown in lane 2. AAV viral proteins VP1, VP2, and VP3 are labelled.

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

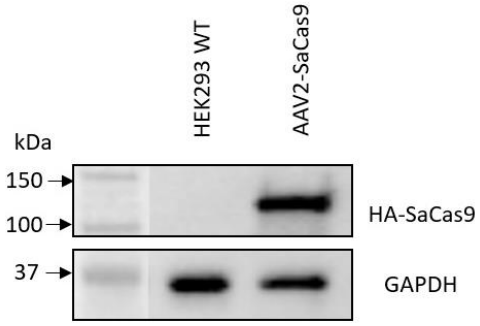

Figure 2. Transduction of HEK293 cells using AAV2 SaCas9.
1 x 10^5 cells were transduced in a 24-well plate with AAV2 SaCas9 at an MOI of 1 x 10^4. After 72 hours of transduction, the expression of HA-tagged SaCas9 in the target cells was measured by Western blot using an Anti-HA.11 Epitope Tag Antibody (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*
AAV2 ZsGreen	78444	50 µl x 2
AAV5 ZsGreen	78447	50 µl x 2
AAV2 Luciferase	78453	50 µl x 2
AAV2 Luciferase-eGFP	78462	50 µl x 2
AAV9 Luciferase-eGFP	78468	50 µl x 2
AAV2 Luciferase-mCherry	78471	50 µl x 2
AAV8 Luciferase-mCherry	78476	50 µl x 2