

Description

Adeno-Associated Virus serotype 8 (AAV8) was first isolated from rhesus monkey tissue, and the AAV8 rep and cap nucleotide sequences have 88% homology with AAV7 and 82% with AAV2. AAV8 exhibits greater transduction efficiency in the liver than other AAV serotypes. AAV8 and 9 have recently been used to correct disease-causing mutations and improve muscle function in mouse models of Duchenne muscular dystrophy.

These AAV8 particles constitutively express the firefly (*Photinus pyralis*) luciferase and enhanced Green Fluorescent Protein (eGFP) genes connected via a T2A linker, under the control of a CMV promoter. The T2A self-cleaving peptide (derived from *Thosea asigna* virus 2A) leads to the efficient cleavage of the transcript, and expression of luciferase and eGFP as two separate proteins.

Application(s)

- Use as a positive control for transduction
- Optimize transduction assays and track protein expression over time

Serotype

Wild-type AAV Serotype 8

Formulation

AAV8 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.

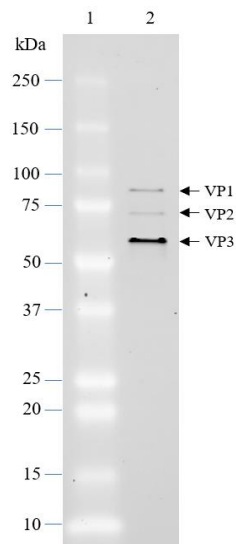


Figure 1. Purified AAV8 Luciferase-eGFP particles.

Staining of a 4-20% SDS-PAGE gel. The protein ladder in lane 1, and 2×10^9 GC (genome copy number) of AAV8 is shown in lane 2. AAV viral proteins VP1, VP2, and VP3 are labeled.

Titer

Two vials (50 μ l x 2) of AAV at a titer $\geq 1 \times 10^{12}$ TU/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.

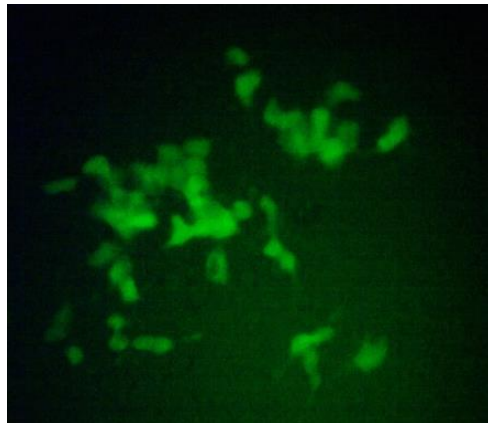
Validation Data

Figure 2. Transduction of HEK293 cells using AAV8 Luciferase-eGFP particles.

1×10^5 cells/well were transduced in a 6-well plate with AAV8 Luciferase-eGFP at an MOI of 2×10^4 . After 72 hours of transduction, eGFP expression in the target cells was observed under a fluorescence microscope. eGFP expression was stable over time and still observed 30 days after transduction.

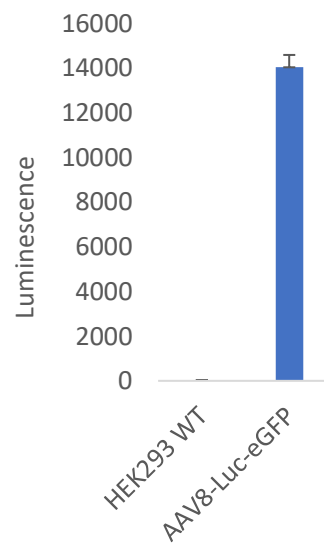


Figure 3. Luciferase activity of HEK293 cells transduced by AAV8 Luciferase-eGFP- particles. 1 x 10⁵ cells/well were transduced in a 6-well plate with AAV8 Luciferase-eGFP at an MOI of 2 x 10⁴. After 72 hours of transduction, transduced cells or parental HEK293 cells were seeded in a 96-well plate at a density of 2 x 10⁴ cells/well, and luciferase activity was measured using the ONE-Step™ luciferase assay system (BPS Bioscience #60690)

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 |
|------------------------|-----------|-----------|
| AAV1 ZsGreen | 78443 | 50 µl x 2 |
| AAV2 ZsGreen | 78444 | 50 µl x 2 |
| AAV5 ZsGreen | 78447 | 50 µl x 2 |
| AAV6 ZsGreen | 78448 | 50 µl x 2 |
| AAV8 ZsGreen | 78449 | 50 µl x 2 |
| AAV9 ZsGreen | 78450 | 50 µl x 2 |
| AAV-DJ ZsGreen | 78442 | 50 µl x 2 |
| AAV5-Luciferase-eGFP | 78645 | 50 µl x 2 |
| AAV6-Luciferase-eGFP | 78666 | 50 µl x 2 |
| AAV9-Luciferase-eGFP | 78468 | 50 µl x 2 |
| AAV-DJ Luciferase-eGFP | 78460 | 50 µl x 2 |