

### 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.

These AAV particles constitutively express the firefly (*Photinus pyralis*) luciferase and mCherry 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 mCherry 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 3

### Formulation

AAV3 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.

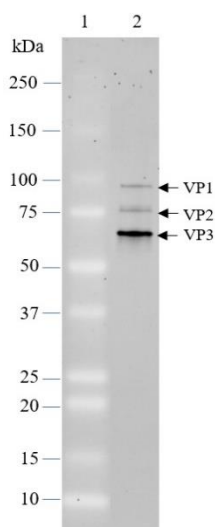


Figure 1. Purified AAV3 Luciferase-mCherry particles.

Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and  $2 \times 10^9$  VG (vector genome) of AAV3 is shown in lane 2. AAV viral proteins VP1, VP2, and VP3 are labeled.

**Titer**

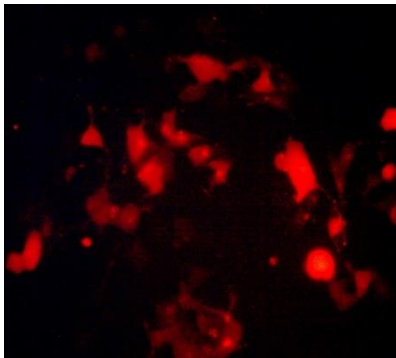
Two vials (50  $\mu$ l x 2) of AAV at a titer  $\geq 1 \times 10^{12}$  vector genomes/ml. The titer is determined by qPCR and will vary with each lot; the exact value will be provided with each shipment.

**Storage**

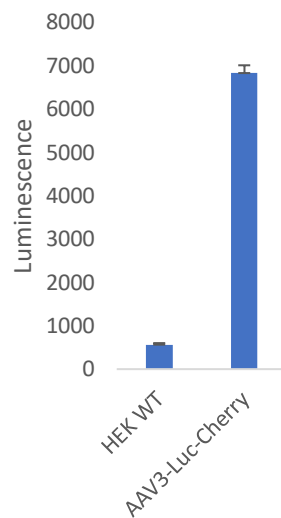
AAV is shipped with dry ice. For long-term storage, it is recommended to store AAV at  $-80^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Titters can drop significantly with each freeze-thaw cycle.

**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 Luciferase-mCherry particles.  $1 \times 10^5$  cells/well were transduced in a 6-well plate with AAV3 Luciferase-mCherry at an MOI of  $2 \times 10^4$ . After 72 hours of transduction, mCherry expression in the target cells was observed under a fluorescence microscope. mCherry expression was stable over time and still observed 30 days after transduction.*



*Figure 3. Luciferase activity of HEK293 cells transduced by AAV3 Luciferase-mCherry particles.  $1 \times 10^5$  cells/well were transduced in a 6-well plate with AAV3 Luciferase-mCherry at an MOI of  $2 \times 10^4$ . After 72 hours of transduction, transduced cells or parental HEK293 cells were seeded in a 96-well plate at a density of  $2 \times 10^4$  cells/well, and luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690)*

### Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://bpsbioscience.com/lentivirus-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

### Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
AAV1 ZsGreen	78443	50 $\mu$ l x 2
AAV2 ZsGreen	78444	50 $\mu$ l x 2
AAV3 ZsGreen	78445	50 $\mu$ l x 2
AAV6 ZsGreen	78448	50 $\mu$ l x 2
AAV3 Luciferase-eGFP	78463	50 $\mu$ l x 2
AAV8 Luciferase-eGFP	78467	50 $\mu$ l x 2
AAV1 Luciferase-mCherry	78470	50 $\mu$ l x 2
AAV8 Luciferase-mCherry	78476	50 $\mu$ l x 2