Description
Adeno-Associated Virus Serotype 3 (AAV3) shares 82% sequence homology with AAV2, and like AAV2, requires Heparan Sulfate Proteoglycan (HSPG) for cellular 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 domain and the intracellular kinase domain of hHGFR are required for AAV3-mediated transgene expression.

These AAV3 particles constitutively express the firefly (*Photinus pyralis*) luciferase and 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 expression over time

Serotype
Wild-type AAV Serotype 3

Formulation
AAV was produced in HEK293-AAV cells and is supplied in PBS-MK (PBS Magnesium-Potassium) buffer with 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.

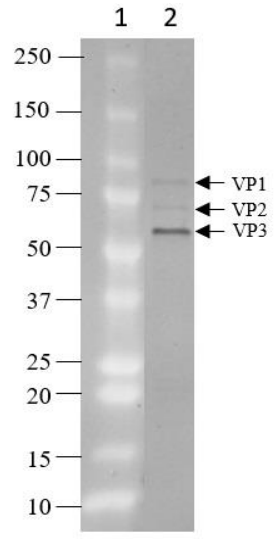

Figure 1. Purified AAV3 Luciferase-eGFP particles.
Staining of a 4-20% SDS-PAGE gel. The protein ladder is in lane 1, and 2 x 10^9 VG (vector genome) of AAV was loaded in lane 2. AAV viral proteins VP1, VP2, and VP3 are labelled.

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

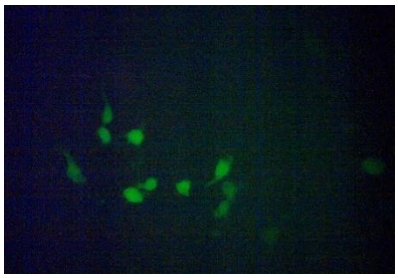

Figure 2. Transduction of HEK293 cells using AAV3 Luciferase-eGFP particles.
1 x 10^5 cells were transduced in a 6-well plate with AAV3 Luciferase-eGFP particles at an MOI of 2 x 10^4. After 72 hours of transduction, eGFP expression in the target cells was observed under a fluorescence microscope. eGFP expression was stable over time and still observed 30 days after transduction.

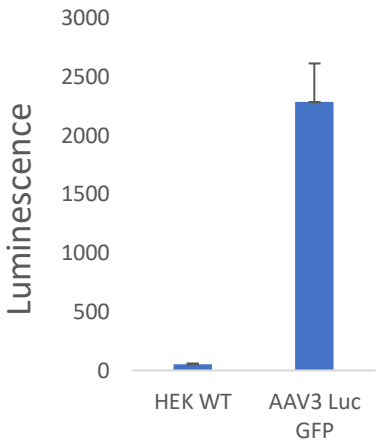

Figure 3. Luciferase activity of HEK293 cells transduced by AAV3 Luciferase-eGFP particles. 1 x 10^5 cells/well were transduced in a 6-well plate with AAV3 Luciferase-eGFP at an MOI of 2 x 10^4. After 72 hours of transduction, transduced cells or parental HEK293 cells were seeded in a 96-well plate at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

Troubleshooting Guide

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
AAV3 ZsGreen	78445	50 µl x 2
AAV5 ZsGreen	78447	50 µl x 2
AAV1 Luciferase-eGFP	78461	50 µl x 2