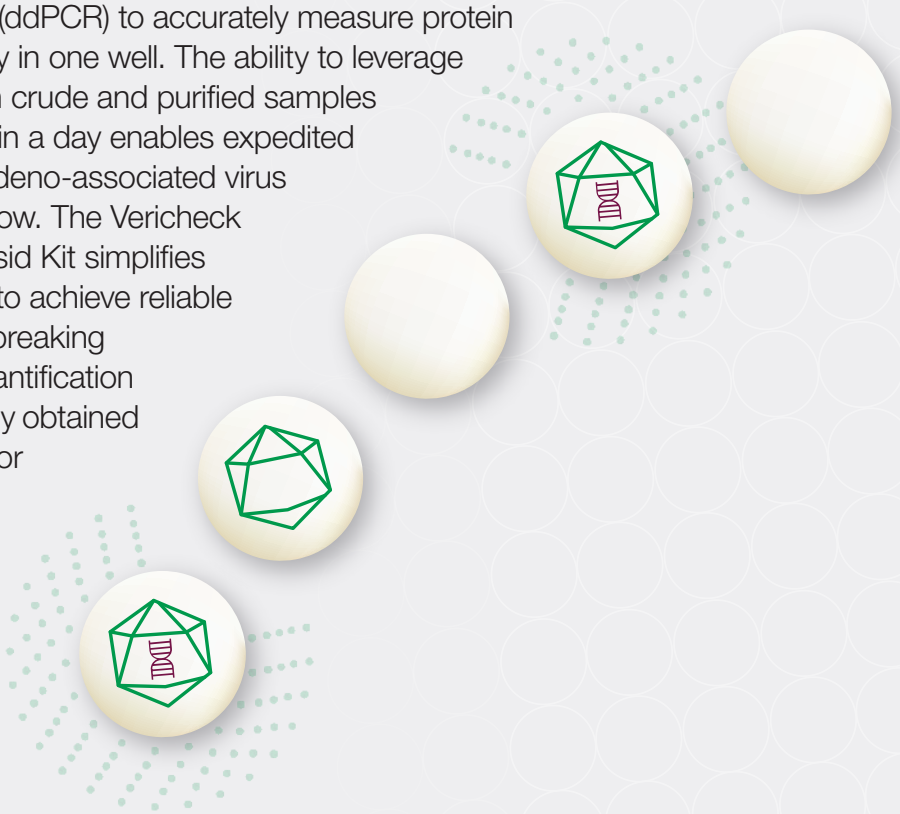
Droplet Digital PCR

Vericheck ddPCR™ Empty-Full Capsid Kit

Achieving optimal therapeutic outcomes in gene therapy relies on precise viral vector characterization. The empty-to-full capsid ratio is a critical quality attribute (CQA) that influences the efficacy and safety of gene therapy treatments. Bio-Rad's Vericheck ddPCR Empty-Full Capsid Kit uses the unparalleled precision of Droplet Digital™ PCR (ddPCR) to accurately measure protein and DNA simultaneously in one well. The ability to leverage the same assay for both crude and purified samples and provide results within a day enables expedited answers for the entire adeno-associated virus (AAV) production workflow. The Vericheck ddPCR Empty-Full Capsid Kit simplifies your AAV development to achieve reliable results with this groundbreaking innovation. Absolute quantification can be easily and quickly obtained via ddPCR technology for various AAV serotypes.



High Precision

High precision of as low as 2% CV*

* CV, coefficient of variation.

Easy Data Analysis

Capsid and genome measurement via ddPCR technology

Fast Turnaround Time

Faster and higher throughput of 8 hr/14 samples

Low Sample Input

Minimal sample input of 2 µl

Multi-Sample Compatibility

For crude lysate and purified samples

Visit **bio-rad.com/VericheckAAV** for more information.

BIO-RAD

Method Overview

The Vericheck ddPCR Empty-Full Capsid Kit method streamlines testing by integrating two familiar techniques: immunochemistry and PCR. The kit adds flexibility to testing by accommodating both crude lysate and purified samples. From a single assay, the kit generates a wealth of data, including vector genome titer, capsid titer, and the percentage of full capsids (Figure 1).

1. **Exogenous DNA removal:** DNase is used to remove interfering DNA outside capsids.
2. **Binding and ligation:** Antibody probes with tethered oligos bind to AAV capsids. Oligos in close proximity ligate together.
3. **Droplet generation and amplification:** Droplets are generated, and the ligated oligo pairs are amplified and detected with ddPCR technology. The ddPCR AAV ITR-2 assay simultaneously detects inverted terminal repeats (ITRs) to identify the AAV genome.
4. **Detection and analysis:** After absolute quantification of capsids and genomes, the empty-to-full ratio is generated.

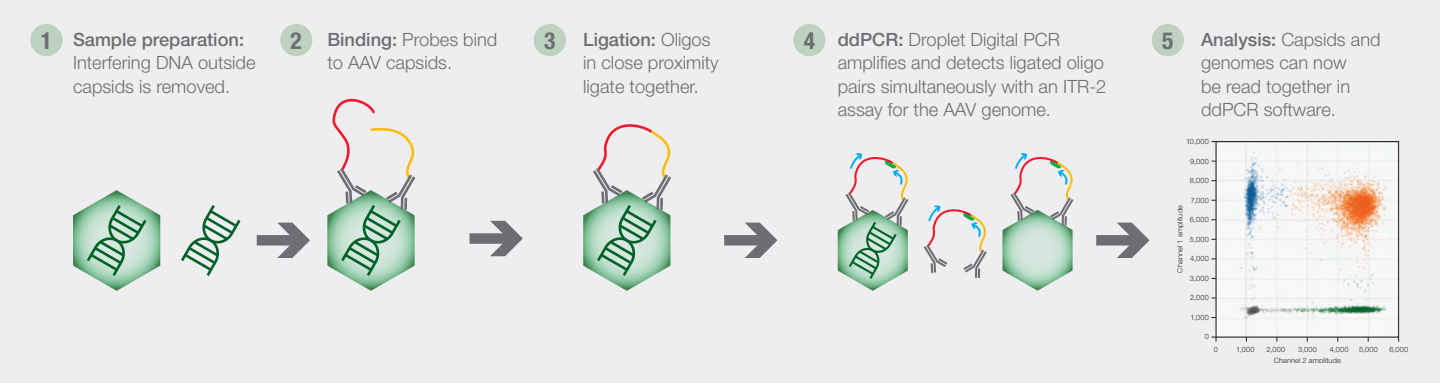


Fig. 1. The Vericheck ddPCR Empty-Full Capsid Kit method. DNase treatment removes external DNA. Probes then specifically bind to AAV capsids. Oligos on these probes ligate together when in close proximity. ddPCR technology then amplifies and detects the ligated oligo pairs, while an ITR-2 assay simultaneously detects the AAV genome. Software analyzes the signals from each capsid type to calculate the empty-to-full capsid ratio.

How ddPCR Technology Quantifies the Empty-Full Capsid Ratio

The Vericheck ddPCR Empty-Full Capsid Kit is a duplex assay that uses fluorescence detection in two channels: FAM and HEX. During PCR, FAM-labeled probes bind to capsid oligos, while HEX-labeled probes bind to vector genome. As a result, droplets fall into one of four distinct fluorescence clusters (Figure 2).

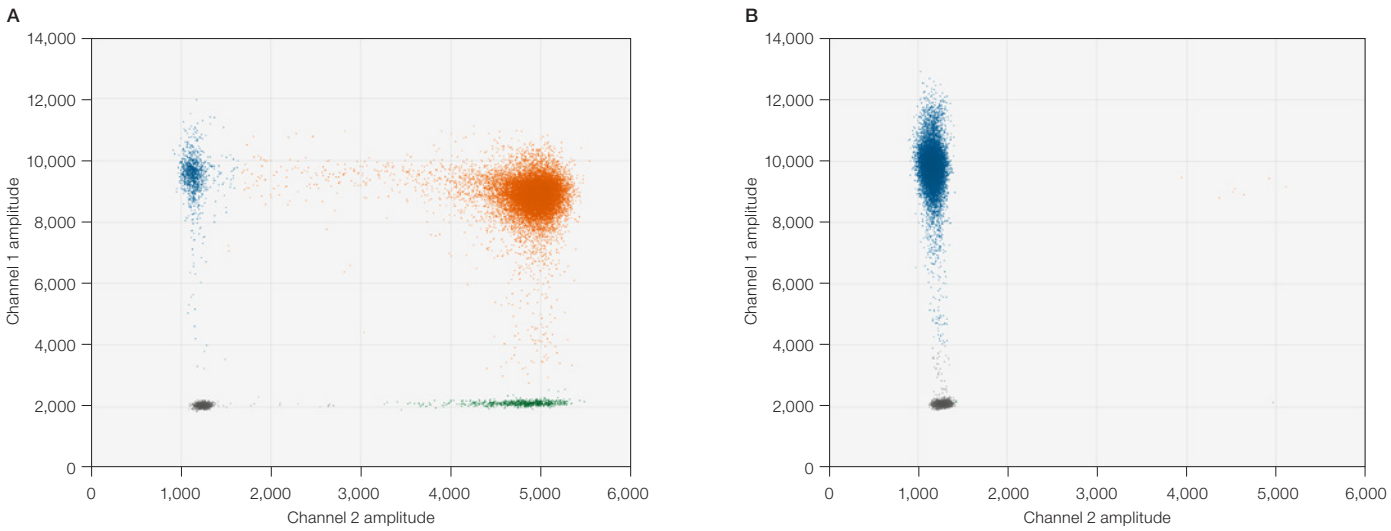


Fig. 2. 2-D amplitude plot for comprehensive ddPCR analysis. The two-channel assay detects fluorescent signals from FAM and HEX dyes. Results are grouped into four main clusters: blue for empty AAV capsid signal (●), orange for full AAV capsid contents containing therapeutic genes (●), gray for negative droplets (●), and green for genome signals (●). **A**, full AAV capsid sample; **B**, empty AAV capsid sample.

High Precision Regardless of Sample Type with ddPCR Technology

Crude lysate and purified samples from any touch point during AAV production are compatible with the Empty-Full Capsid Kit. With only 2 µl of sample required,* the kit allows for high precision of major AAV serotypes (Figure 3).

* Concentration range: 10^9–10^{12} viral genomes per ml (vg/ml) or capsids per ml (cp/ml).

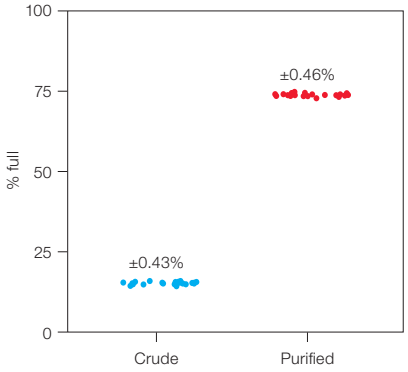


Fig. 3. Low coefficient of variation (CV) for AAV serotype 9 (AAV9). The Vericheck ddPCR Empty-Full Capsid Kit was used to evaluate crude cell lysate and purified AAV. The results demonstrate consistent and precise measures of the percentage of full capsids. Note that no additional steps are required to measure crude samples. Crude samples (●); purified samples (●).

Highly Reproducible with a Shorter Turnaround Time

The Vericheck ddPCR Empty-Full Capsid Kit provides highly repeatable results within 8 hours across multiple samples, users, lots, instruments, and days (Figure 4).

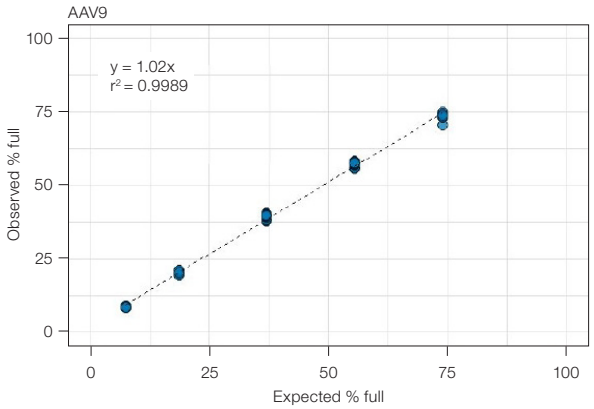


Fig. 4. Repeatability across the measuring range. An AAV sample with predominantly filled capsids was mixed with empty capsids in varying ratios to create five samples across the measuring range. Each point represents a single test, with the dotted line indicating the linear regression analysis. The results show a consistent linear relationship between the expected and observed percentages of full capsids, from low to high levels, with high precision maintained throughout the range.

Higher Accuracy with ddPCR Technology

Recent studies have found that contaminant DNA encapsulated within AAVs may be counted as full capsids in analytical ultracentrifugation (AUC) but not in ddPCR methods (Lecomte et al. 2015, McColl-Carboni et al. 2024, Schnödt et al. 2016). Droplet Digital PCR quantifies the amount of viral DNA within particles by amplifying specific genomic regions. Specifically, ddPCR technology provides information on genome titer, capsid titer, and the percentage of full capsids — measured in a single test without external standard curves. In contrast, AUC separates particles by sedimentation rates, providing detailed physical characterization (for example, size, shape, and mass) without a reference standard (Figure 5).

AUC	**Full**	**Partial**	**Empty**	**Heavy**	**Full**
ddPCR	**Full**	**Partial**	**Empty**	**Not detected**	**Empty**

Fig. 5. With ddPCR technology, only ITR-2 is detected, not contaminants, ensuring accurate categorization of full capsids. Analytical ultracentrifugation (AUC) differentiates empty and full capsids by mass, whereas the ddPCR method is specific to capsid content. Content-blind methods like AUC overestimate the number of therapeutic capsids by counting contaminants as full. As shown, capsids containing residual plasmid DNA are categorized full by AUC but negative by the ddPCR method. Additional residual DNA from host cell DNA may account for further differences.

Performance of ddPCR versus Other Technologies

The ddPCR method streamlines AAV development with its high precision and ability to process crude lysate and purified samples. Measurement of the empty-to-full ratio can be performed at any point in AAV production with a sample input as low as 2 µl (Figure 6).

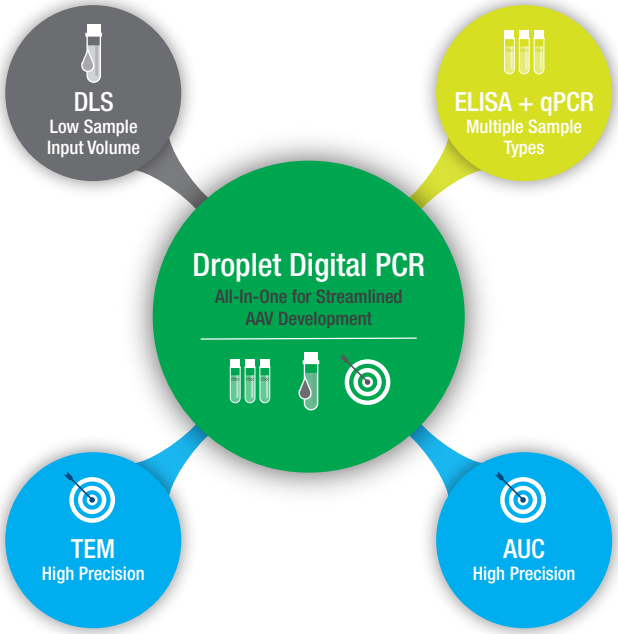


Fig. 6. Unlike other methods in AAV quantification and characterization, ddPCR technology offers an all-in-one solution for streamlining AAV development by delivering high-precision measurements from minimal sample volumes. Dynamic light scattering (DLS) enables low sample volume analysis, enzyme-linked immunosorbent assay (ELISA) with qPCR supports various sample types, and transmission electron microscopy (TEM) and analytical ultracentrifugation (AUC) provide high-precision analysis. By integrating all these capabilities in one technology, Droplet Digital PCR simplifies AAV development workflows.

Kits

Vericheck ddPCR Empty-Full Capsid Kit (AAV5)

Vericheck ddPCR Empty-Full Capsid Kit (AAV9)

References

Lecomte E et al. (2015). Advanced characterization of DNA molecules in rAAV vector preparations by single-stranded virus next-generation sequencing. Mol Ther Nucleic Acids 4:e260.

McColl-Carboni A et al. (2024). Analytical characterization of full, intermediate, and empty AAV capsids. Gene Ther 31, 285–294.

Schnödt M et al. (2016). DNA minicircle technology improves purity of adeno-associated viral vector preparations. Mol Ther Nucleic Acids 5:e355.

Ordering Information

Catalog #	Description
17010072	**Vericheck Empty-Full Capsid AAV5 Kit**
17010082	**Vericheck Empty-Full Capsid AAV9 Kit**

Visit bio-rad.com/VericheckAAV for more information.

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