Expedited quantification of AAV titers using a single-wash ELISA

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ABSTRACT

Adeno-associated viruses (AAVs) serve as essential vectors in gene therapy and biomedical research, requiring precise and efficient titer quantification methods to ensure experimental consistency and therapeutic success. This study presents optimized single-wash enzyme-linked immunosorbent assays (ELISAs) for rapid, reproducible quantitation of AAV titers for serotypes 2, 8, and 9.

A key attribute of these assays is the single-wash protocol, which minimizes hands-on time and procedural complexity while preserving analytical rigor. The streamlined workflow is completed within 90 min and enables compatibility with automation systems, delivering significant time savings compared to conventional ELISA methods. Validation studies demonstrated a high degree of concordance with quantitative PCR (qPCR) titer data, underscoring the reliability of the streamlined workflow.

The assays employ highly specific antibodies detecting AAV capsid proteins for accurate viral particle capture. A horseradish peroxidase (HRP)-conjugated antibody for AAV detection allows for signal generation through enzymatic activity. Empty AAV particles of each serotype with known titers are used to create the standard curve for quantitation. This approach achieves a linear detection range of 10^7 to 10^9 viral particles/ml (vp/ml) and allows for a limit of detection (LOD) of 1.10×10^7 vp/ml and a limit of quantitation (LOQ) of 3.63×10^7 vp/ml. The assays provide a high level of consistency, with inter-assay and intra-assay coefficients of variation (CV) below 20% and 10%, respectively.

These single-wash assays offer a rapid, sensitive, and reproducible method for AAV titer quantification. Its optimized protocol and validated accuracy make it a valuable tool for advancing AAV-based gene therapy research and production, addressing the field's growing demand for efficient and reliable methodologies for AAV quantification.

1 Principle

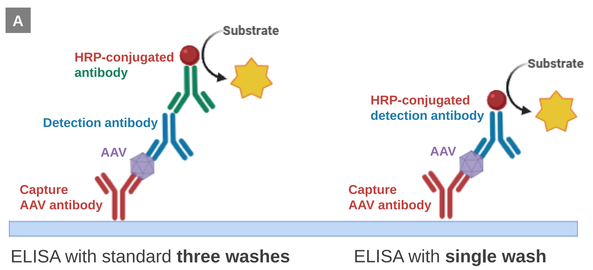


Figure 1. Principle behind AAV Rapid Titer ELISA Kit (Single Wash). Panel A. Unlike a standard three-wash ELISA (left), a single-wash ELISA (right) utilizes analyte directly mixed with an HRP-conjugated detection antibody. **Panel B.** Workflow overview for AAV Rapid Titer ELISA Kit (Single Wash). AAV antibodies, coated on the bottom of the ELISA plate, capture AAV particles contained in a sample. At the same time, an HRP-labeled AAV detection antibody is added to the mix. After a 1 hr incubation, followed by a single wash, the substrate is added and the signal is read after 15 min. R.T. = room temperature. Created with BioRender.com.

2 Protocol

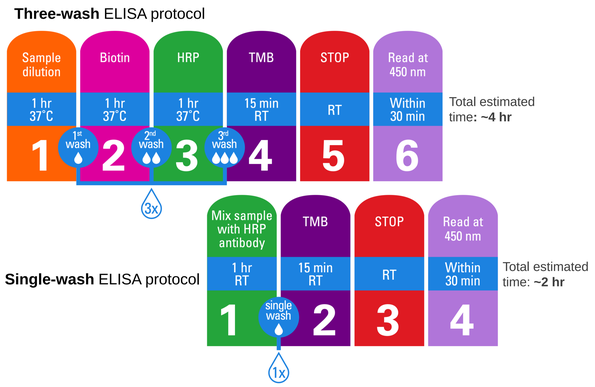


Figure 2. AAV Rapid Titer ELISA Kit (Single Wash) protocol involves fewer overall steps, including only a single wash step. This protocol simplifies the process and reduces hands-on time and effort for titer determination.

3 Benchmarking

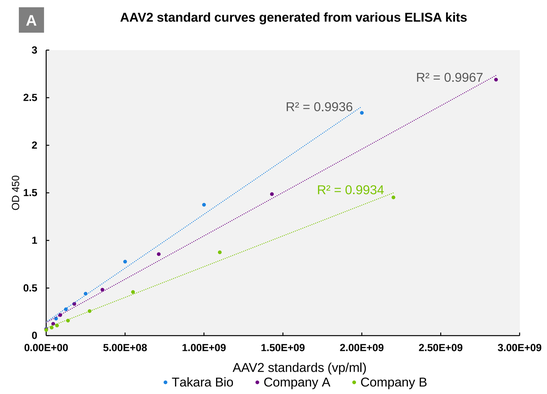
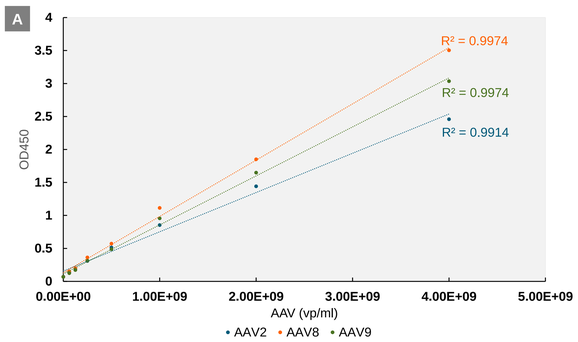


Figure 3. Performance comparison of AAV2 Rapid Titer ELISA Kit (Single Wash) to other ELISA kits. AAV2 Rapid Titer ELISA Kit (Single Wash) was tested against different commercially available ELISA kits using serial dilutions of AAV2 control (**Panel A**) and AAV2-CMV-GFP (Virovek, **Panel B**). The results demonstrate comparable accuracy and efficiency of the AAV2 Rapid Titer ELISA Kit (Single Wash) to more complex protocols, enabling researchers to obtain reliable data with minimal steps and enhancing laboratory productivity.

4 Performance



B

Parameters	
R^2	>0.99
LOD	1.10×10^7
LOQ	3.63×10^7
Intra-assay CV	<10%
Inter-assay CV	<20%

Figure 4. The AAV ELISA Rapid Titer Kit (Single Wash) generates consistent and reproducible results for viral titers. Panel A. Representative linearity curves for AAV2, AAV8, and AAV9. Standard dilutions were prepared using AAV2, AAV8, and AAV9 empty particles and assayed with corresponding AAV Rapid Titer ELISA Kits (Single Wash). R^2 values are based on linear regression analysis. The strong linear correlation demonstrates the assay's accuracy and reliability for quantifying AAV across a range of concentrations. **Panel B.** Table of parameters for linearity curves.

5 Compatibility with different sample matrices

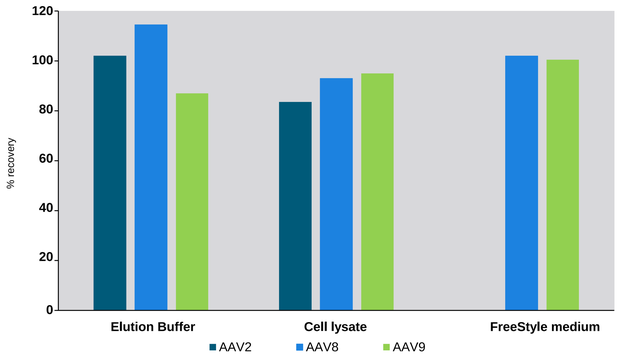


Figure 5. AAV ELISA Rapid Titer Kit (Single Wash) is compatible with multiple sample matrices. To simulate various sample conditions, 2 x 10^9 control AAV particles were spiked into 1 ml of different matrices: (1) Elution Buffer from the AAVpro® Cell Purification Kit Maxi (All Serotypes) (Takara Bio, Cat. # 6666), (2) 293 Cell Lysate, and (3) FreeStyle F17 Expression Medium (Gibco, Cat. # A1383501). All samples were tested using AAV ELISA Rapid Titer Kit (Single Wash) for AAV2, AAV8, and AAV9. The data demonstrate the compatibility of the ELISA kit with different sample matrices, ensuring accurate AAV quantification across various preparation conditions.

6 Correlation of viral titers from ELISA, qPCR, and lateral flow assays

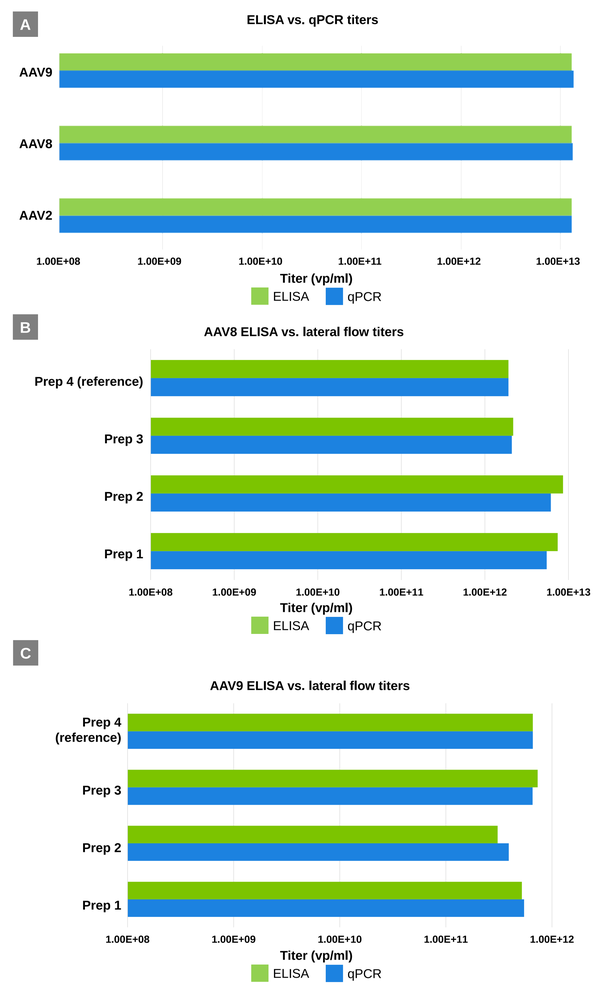


Figure 6. Correlation between AAV quantifications obtained using ELISA, qPCR, and lateral flow assays for different AAV serotypes. Bar graphs comparing titers from the AAV ELISA Rapid Titer Kit (Single Wash) with titers from the AAV Real-Time PCR Titration Kit (Takara Bio, Cat. # 6233) (**Panel A**) or lateral flow assays (**Panels B & C**). Preparation ("Prep") 4 was used as a reference to determine the ELISA-equivalent value from lateral flow readings. The strong correlation of results across these three methods demonstrates the reliability of AAV ELISA Rapid Titer Kit (Single Wash) and establishes it as a robust, rapid, and convenient method for researchers to measure viral titers.

7 Automation

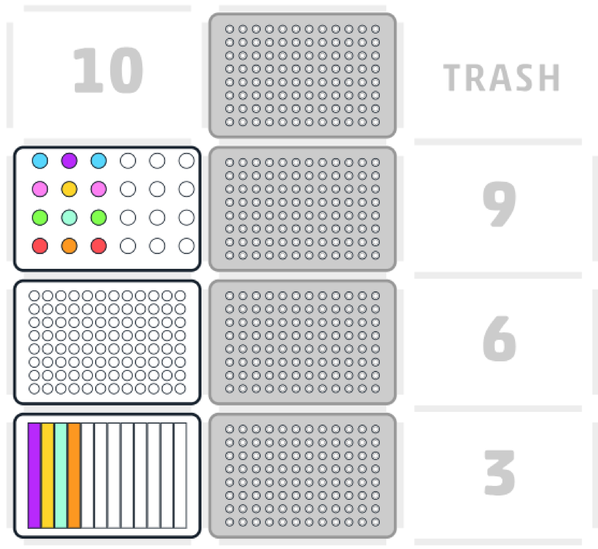


Figure 7. Deck layout of a high-throughput liquid handling robot (Opentrons Labworks) for quantifying AAV titer using AAV ELISA Rapid Titer Kit (Single Wash). The unified protocol supports simultaneous quantification of serotypes AAV2, AAV8, and AAV9.

CONCLUSIONS

Simplified workflow
The single-wash protocol of AAV ELISA Rapid Titer Kit (Single Wash) reduces steps and hands-on time while maintaining accuracy.

High accuracy and reliability
AAV ELISA Rapid Titer Kit (Single Wash) demonstrates strong linear correlation across standard dilutions for AAV2, AAV8, and AAV9 serotypes, with high R^2 values.

Broad sample compatibility
AAV ELISA Rapid Titer Kit (Single Wash) maintains its performance across various sample matrices—including cell lysates, elution buffers, and expression media.

Comparable to qPCR
The titers of AAV samples determined with the AAV ELISA Rapid Titer Kit (Single Wash) strongly correlate with those obtained from qPCR-based methods, validating the kit as a rapid and reliable alternative to qPCR for AAV quantification.

Automation and scalability
The single-wash protocol simplifies the integration of ELISA into automated platforms, enabling simultaneous quantification of AAV2, AAV8, and AAV9 through an automated liquid-handling workflow.

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