

# Fast and Reproducible AAV Vector Quantification with Simple Plex Assays on Ella

## Introduction

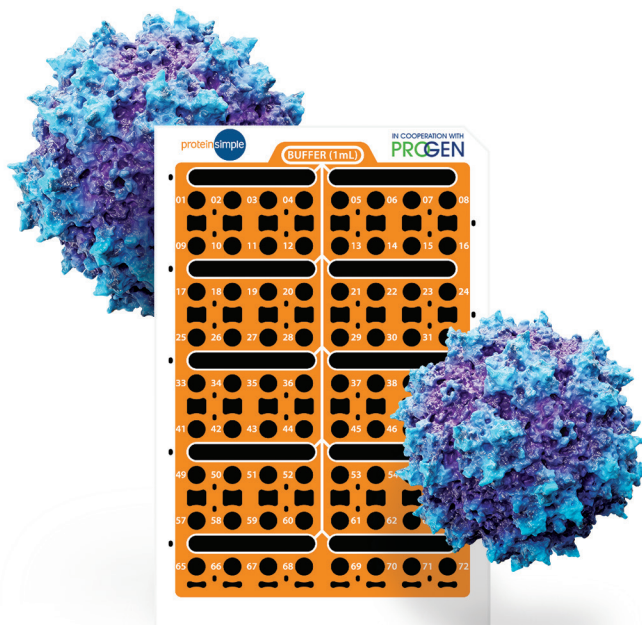
As part of the viral quantification process, researchers rely on various analytical methods to assess critical quality attributes. While traditional plate-based and dot blot assays can be useful, they are often complex, time-consuming, and non-standardized. To meet increased throughput and safety demands, fast and reproducible titration of viral particles is essential. This growing need has spurred development of more automated analytical solutions, which are paving the way for increases in facility throughput while helping to make quality control faster, more accurate, and more reproducible.

In this application note, we pair Simple Plex™ microfluidic cartridge assays on Ella™ with PROGEN antibodies and demonstrate utility for accurate and efficient AAV capsid titer determination. We show how the superior specificity of PROGEN's extensively characterized serotype specific antibodies helps ensure reliable AAV quantification. We also demonstrate that Simple Plex AAV viral titer assay data is highly correlated with data generated using the corresponding PROGEN AAV Titration ELISA.

## Why Pair Ella with PROGEN AAV Antibodies?

For application of AAV vectors in gene therapy, the determination of total capsid titer is essential. PROGEN AAV Titration ELISAs are well-known for their ability to provide reliable and accurate quantification of AAV total capsid titers, based on the proven sensitivity and specificity of PROGEN AAV antibodies for AAV intact particles. The Simple Plex AAV assays offer the same proven specificity as the industry-leading PROGEN ELISA along with the convenience, sample-conserving, and time-saving advantages inherent in the fully automated Ella platform.

All steps of the Simple Plex AAV assays on Ella are highly automated thanks to the microfluidic cartridge—everything is preloaded, even the calibration curve. Just pipette your diluted samples onto the cartridge, add wash buffer, press start in the Ella Runner Software and walk away to the sound of automatic processing.



Inside the microfluidic cartridge are glass nano reactors (GNRs), the core of the Simple Plex assay. In Simple Plex AAV assays, the GNRs are coated with the PROGEN antibodies (TABLE 1) that work to capture intact AAV capsids. That same corresponding AAV antibody is also lyophilized in the cartridge to act as the detection reagent. Ella's fluorescence detection system enables detection across a broad dynamic range, minimizing the need to run multiple dilutions to find the ideal MRD (see TABLE 1). The efficient cartridge design, featuring three independent GNRs per sample inlet, eliminates the need to run samples in duplicate. In total, from set up to fully processed and analyzed results takes less than 90 minutes.

## Linear Correlation with the PROGEN ELISA

In FIGURE 1, we demonstrate that AAV levels measured by the Simple Plex AAV1, AAV2, AAV6, and AAV8 viral titer assays strongly correlate with those obtained by the corresponding PROGEN AAV Titration ELISAs. With Simple Plex AAV assays running on Ella, users currently utilizing PROGEN AAV ELISAs now have an automated option that preserves data continuity.

Serotype	PROGEN Mouse Monoclonal Antibody	PROGEN ELISA Control	Simple Plex Assay LLOQ (capsids/mL)	Simple Plex Assay ULOQ (capsids/mL)	Logs of Dynamic Range
AAV1	ADK1	PRAAV1-C	2.62 X 10 <sup>6</sup>	1.0 X 10 <sup>10</sup>	~3.5
AAV2	A20	PRAAV2-C	3.41 X 10 <sup>6</sup>	1.3 X 10 <sup>10</sup>	~3.5
AAV6	ADK1	PRAAV6-C	3.04 X 10 <sup>6</sup>	1.16 X 10 <sup>10</sup>	~3.5
AAV8	ADK8	PRAAV8-C	2.47 X 10 <sup>6</sup>	9.428 X 10 <sup>10</sup>	~3.5

Table 1. Simple Plex AAV assays use the same high quality antibodies as PROGEN ELISAs. The Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) are indicated for each assay in capsids per milliliter.

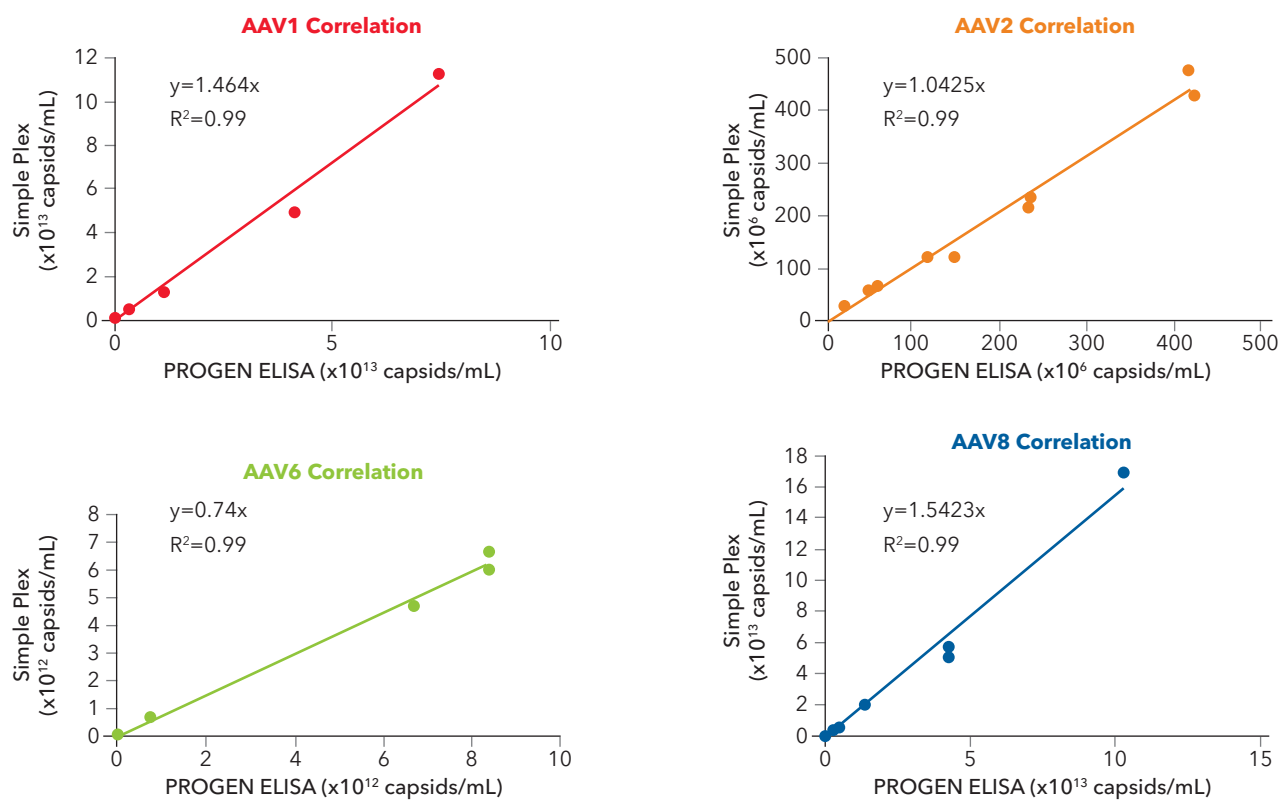


FIGURE 1. Linear correlations of the Simple Plex AAV assays and PROGEN AAV ELISAs. The aggregated Simple Plex and PROGEN ELISA data was plotted and a linear fit was applied to the data for each AAV serotype. The Simple Plex AAV assays are correlated with the corresponding PROGEN ELISAs as follows: AAV1  $R^2=0.99$  (2a), AAV2  $R^2=0.99$  (2b), AAV6  $R^2=0.99$  (2c), AAV8  $R^2=0.99$  (2d). The data shows a nearly perfect correlation where the quantitative range of both assays overlap.

## AAV Spiked Recovery with Simple Plex Assays

To demonstrate assay performance, recovery of spiked samples was tested using two conditioned medias and one cell supernatant. Samples were spiked with a low, medium, or high concentration of recombinant AAV serotypes, which consists of fully assembled empty AAV capsids (FIGURE 2).

## Sample Linearity of Multiple In-Process Pools with Simple Plex AAV Assays

To examine the ability of the Simple Plex AAV assays to accurately quantify AAV particles across viral vector samples and dilution ranges, in-process samples were assessed for linearity across four dilutions. Initial sample dilutions were based on values that were predicted by the PROGEN AAV Titration ELISAs to fall within the linear range of the Simple Plex AAV viral titer assays. All samples were then serially diluted 1:2, 1:4, 1:8, and 1:16. The percent difference between each sample value and the least diluted sample was then calculated.

When taken in aggregate, the sample performance showed good linearity across four dilution points and a large range of incorporated matrix, with all percent recoveries falling between 80–120% (FIGURE 3). This demonstrates that the sensitive nature of PROGEN antibodies translates well to the robust performance of the Simple Plex AAV assay format on Ella. The initial dilution of these samples, as recommended by the PROGEN AAV Titration ELISAs ranged from 1:2 to 1:32.

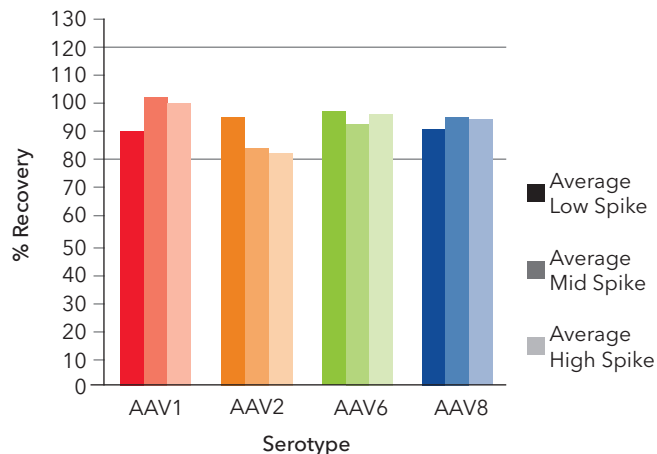


FIGURE 2. To demonstrate assay performance, recovery of spiked samples was tested across conditioned medias. Samples were spiked with a low, medium, or high concentration of relevant AAV which consists of fully assembled empty capsids. To interrogate the breadth of the assay range, we chose the following concentrations: 1:8, 1:80, 1:800 (for AAV1, AAV8), 1:10, 1:00, 1:1000 (for AAV6), and 1:2, 1:50, 1:100 (for AAV2) of the first standard point.

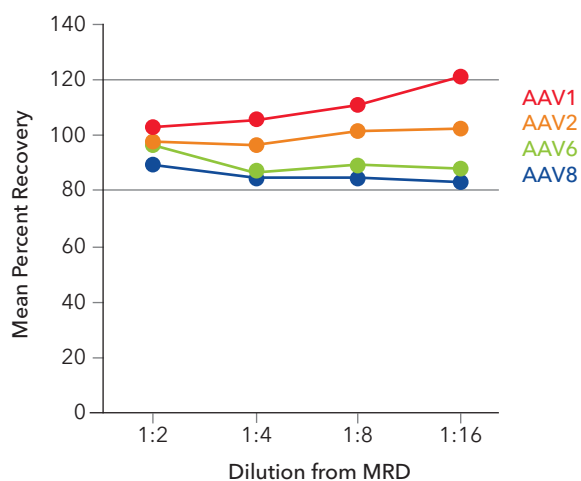


FIGURE 3. Each in-process sample showed good dilutional linearity at all 4 dilutions tested, with 80-120% percent recoveries compared to the minimum required dilution (MRD).

## Simple Plex Assay Precision

Ella’s hands-off AAV quantitation workflow consistently delivers high-quality, reproducible data. To examine assay precision, we used purified AAV particles to assess reproducibility within a cartridge and across many cartridges. Low and high controls were prepared using the following reference materials: viral material (AAV1, AAV6) the PROGEN AAV2 ELISA control and the Reference Standard Material (RSM) from ATCC (AAV2, AAV8).

## Inter-Assay Results

Using low- and high-level controls, replicates were tested across multiple cartridges, cartridge lots and Ella instruments. Low and high controls prepared with either reference material showed inter-assay CV<12%, attesting to Ella’s ability to report the same concentration from separately run cartridges. Across the dynamic range of the assay, the data showed reproducible results with either material (Table 2).

	AAV1	AAV2	AAV6	AAV8
Low Control	8.5%	10.7%	11.5%	9.2%
High Control	6.4%	8.1%	8.0%	4.5%

Table 2. Inter-assay precision. Low and High Control samples all have excellent inter-assay precision. Inter-assay precision was below 12% for all Simple Plex AAV assays.

## Intra-Assay Results

Using low- and high-level controls, replicates were tested per user of each level control. For either material, intra-assay precision was better than 10%. (Table 3).

	AAV1	AAV2	AAV6	AAV8
Low Control	2.8%	8.8%	4.6%	2.9%
High Control	4.1%	3.0%	3.2%	5.2%

Table 3. Intra-assay precision. Low and High Control samples all have excellent intra-assay precision. Intra-assay precision was less than 10% for all Simple Plex AAV assays.

## Conclusion

AAV capsid titer quantification with Simple Plex AAV assays offers a robust alternative to the conventional ELISA or dot blot approaches. Ella is an automated immunoassay platform that eliminates the hands-on steps that come with running a traditional immunoassay. You can significantly decrease time to result and reduce human error, both factors that adversely impact assay reproducibility and team productivity. The Simple Plex analysis software is available in a 21 CFR Part 11

compliant version. These attributes make Ella an ideal tool for industry environments where a good manufacturing practice (GMP) system is in place. All in all, Ella satisfies the demands of timely data generation for maintaining efficient production and overcomes the lengthy investigations and sample retests that often result from human error.

You also get excellent assay sensitivity and highly reproducible results, which helps ensure you get the high-quality data required to meet regulatory standards for AAV titration throughout viral vector production workflow. The broad range of detection achievable with Simple Plex viral titer assays is particularly useful for AAV quantification, as samples need fewer dilutions to fall within the quantitative range of the assay. With Ella, you get an extended quantitative range and improved precision with the same excellent sample performance you get with PROGEN AAV Titration ELISAs.

## Controls and Reference Materials

Be sure to follow manufacturer recommendations for proper storage and handling of Simple Plex AAV2 Assay controls and reference materials.

- ATCC Recombinant Adeno-associated virus 2 (VR-1616) and Adeno-associated virus 8 (AAV8RSS03112010P2) viral reference material is shipped in a liquid/frozen format. Recommended storage (in the vapor phase of liquid nitrogen) is at -70°C or colder. Material can be diluted in SD19 diluent for multi-level controls.

**Detailed product and ordering info online at**  
[www.atcc.org/products/vr-1616](http://www.atcc.org/products/vr-1616)

- The PROGEN ELISA controls (See TABLE 1) consist of fully assembled, empty AAV capsids. Product is shipped as a lyophilized/cold pack. Recommended storage is between 2 and 8 °C. Material is sensitive to vortexing, freeze-thaw cycles, and storage at low concentrations. Material can be diluted in SD19 diluent for multi-level controls.

**Detailed product and ordering info online at**  
[us.progen.com/AAV/AAV-ELISA/All-AAV-Control-Products/](http://us.progen.com/AAV/AAV-ELISA/All-AAV-Control-Products/)

## Sample Dilution Guidance

Testing should be performed to identify a sample dilution that produces acceptable performance and accurate quantitation. Due to unique sample matrices inherent in viral vector production, we recommend testing each unique matrix for dilutional linearity and spike recovery at several sample dilution levels. Linear dilution should result in back-calculated values consistent with the value derived from the least diluted sample (+/-20%). Recombinant AAV spiked into diluted matrix should recover between 80-120% when compared to the same material spiked into sample diluent.

## Matrix Effects of Additives

The determination of AAV capsid titers from cell extract can be influenced by several conditions such as the composition of your lysis buffer. For example, high concentrations of certain components in your buffer might inhibit adequate capsid detection.

**Learn about potential matrix effects at PROGEN**  
[us.progen.com/AAV/AAV-ELISAs](http://us.progen.com/AAV/AAV-ELISAs)

## Related Resources

Simple Plex assays are available for a variety of applications including Lentiviral titration, bioprocess testing, cancer and cell therapy research and development.

- [Viral Titer Quantification](#)
- [Bio-Process Testing](#)
- [Cancer](#)
- [Cell Therapy](#)



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