



Introduction

Adeno-associated viruses (AAVs) have been a potent tool for gene delivery. Canonical AAV serotypes and engineered AAV variants exhibiting a wide array of cell and tissue tropisms are employed for various therapeutic applications. Accurate AAV capsid titer determination is vital for the clinical application of AAVs. Yet, isolating high-quality monoclonal antibodies (MAbs) specific to AAV serotypes for titer determination is challenging due to AAV's weak immunogenicity. To overcome this, KACTUS have developed an efficient mouse AAV immunization scheme, leading to strong antibody responses and efficient antibody isolation. Based on this, we have successfully developed high-quality AAV Titration ELISA Kits against different AAV serotypes. Moreover, our platform technology can also be extended for the customization of ELISA kits against engineered AAV capsids.

Background

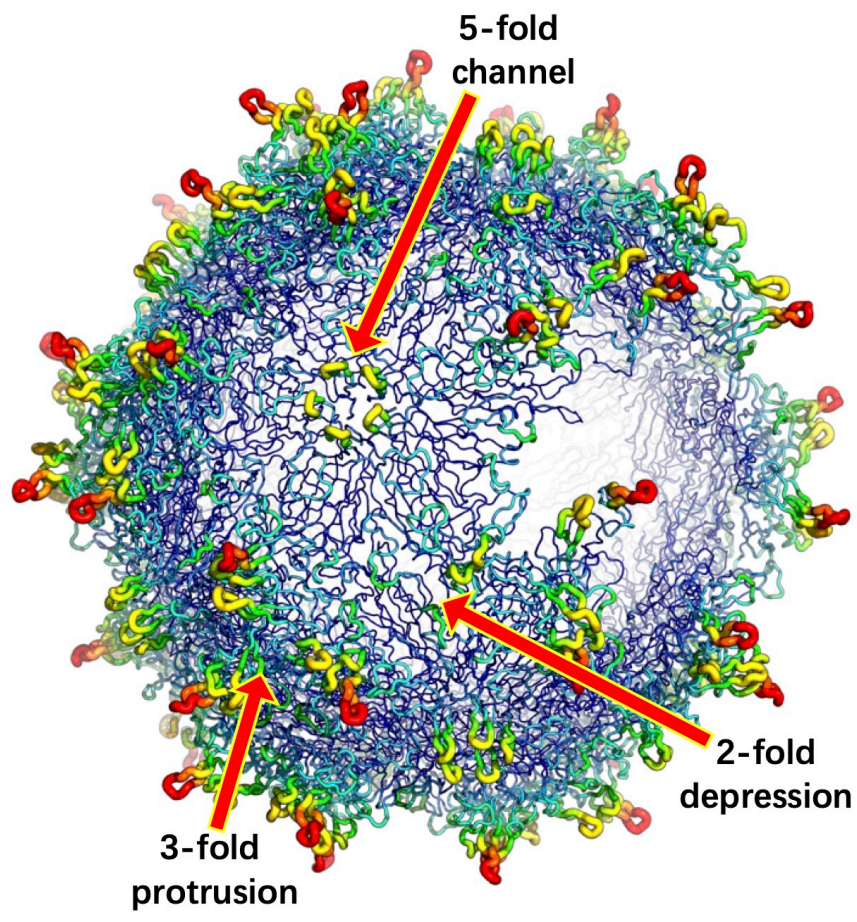


Figure 1. The overall structural characteristics of the AAV particle.

Despite variations in serotypes, all AAV capsids are composed of 60 copies of three capsid viral proteins (VP1/VP2/VP3), which assemble into an icosahedron in an approximate molar ratio of 1:1:10 (VP1:VP2:VP3). These three VP proteins differ at the N-terminus, with the complete VP3 sequence nested within VP2, which, in turn, is contained within VP1.

The structures of AAV capsids exhibit distinct features, including 5-fold channels, 3-fold protrusions, and 2-fold depressions. Typically, the 3-fold protrusions contain essential epitopes for highly specific antibodies (Fig. 1). Most of these epitopes overlap with receptor-binding sites on the capsid, making them the primary target for AAV variant engineering.

Self-Developed High-Quality mAbs Against Specific AAV Serotypes

KACTUS patented immunization method has proven to be a more efficient scheme to obtain antibodies against AAVs. Utilizing this innovative approach, we successfully procured a serum antibody with an elevated titer (Fig. 2A). Based on this, we screened and isolated recombinant monoclonal antibodies against AAV2, AAV5, AAV6, AAV8, and AAV9. These monoclonal antibodies recognize intact viral capsids without binding to denatured capsids (Fig.2B). Furthermore, these antibodies demonstrate high specificity, with no cross-reactivity observed to other serotypes (Fig. 2C).

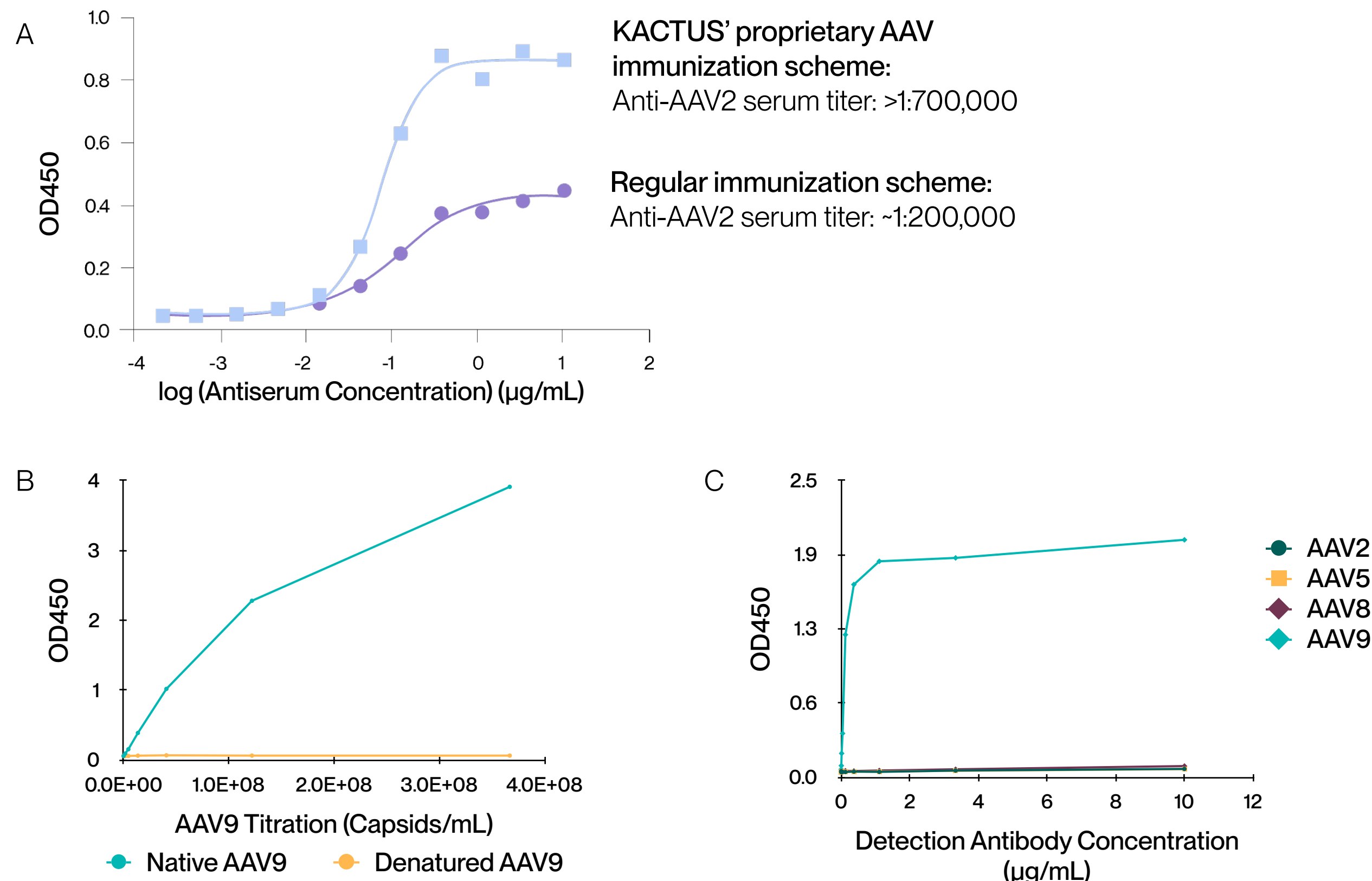


Figure 2. KACTUS patented immunization method for AAV antibody discovery and development. (A) Comparison of anti-AAV2 serum titers for the KACTUS proprietary versus standard immunization protocols. (B) Binding of AAV9 antibodies to intact and denatured AAV9 capsid proteins using ELISA sandwich assay. (C) Evaluation of cross-binding of AAV9 antibody to other serotypes (AAV2/5/8/9). The recombinant anti-AAV9 monoclonal antibody developed from our proprietary immunization scheme is highly specific to AAV9 intact capsids and does not exhibit cross-reactivity with other AAV serotypes.

High Performance of AAV Titration ELISA Kits

Leveraging our proprietary anti-AAV antibody discovery platform, KACTUS has developed a series of off-the-shelf AAV Titration ELISA kits for various serotypes, as well as custom AAV kit development for engineered capsids. These kits feature a wide linear range, high intra-assay precision and recovery rate, and long-term stability.

Catalog No.	Product Title
AV2-MM00B	AAV2 Titration ELISA Kit
AV5-MM00B	AAV5 Titration ELISA Kit
AV6-MM00B	AAV6 Titration ELISA Kit
AV8-MM00B	AAV8 Titration ELISA Kit
AV9-MM00B	AAV9 Titration ELISA Kit

Table 1. AAV Titration ELISA Kit products by KACTUS.

Wider Linear Range: Enhancing Measurement Accuracy

The wide linear range of the kits allow for accurate detection of samples across a broader spectrum of concentrations. Since the characteristics of the mAbs directly impact the kit's performance, we selected mAbs with extended detection ranges during the antibody screening process for the development of the KACTUS AAV titration kits. Here, we compare the performance of our kits to another leading supplier. The results show KACTUS AAV Titration ELISA Kits have a wider linear range and a better signal-to-noise ratio (Figure 3, 4).

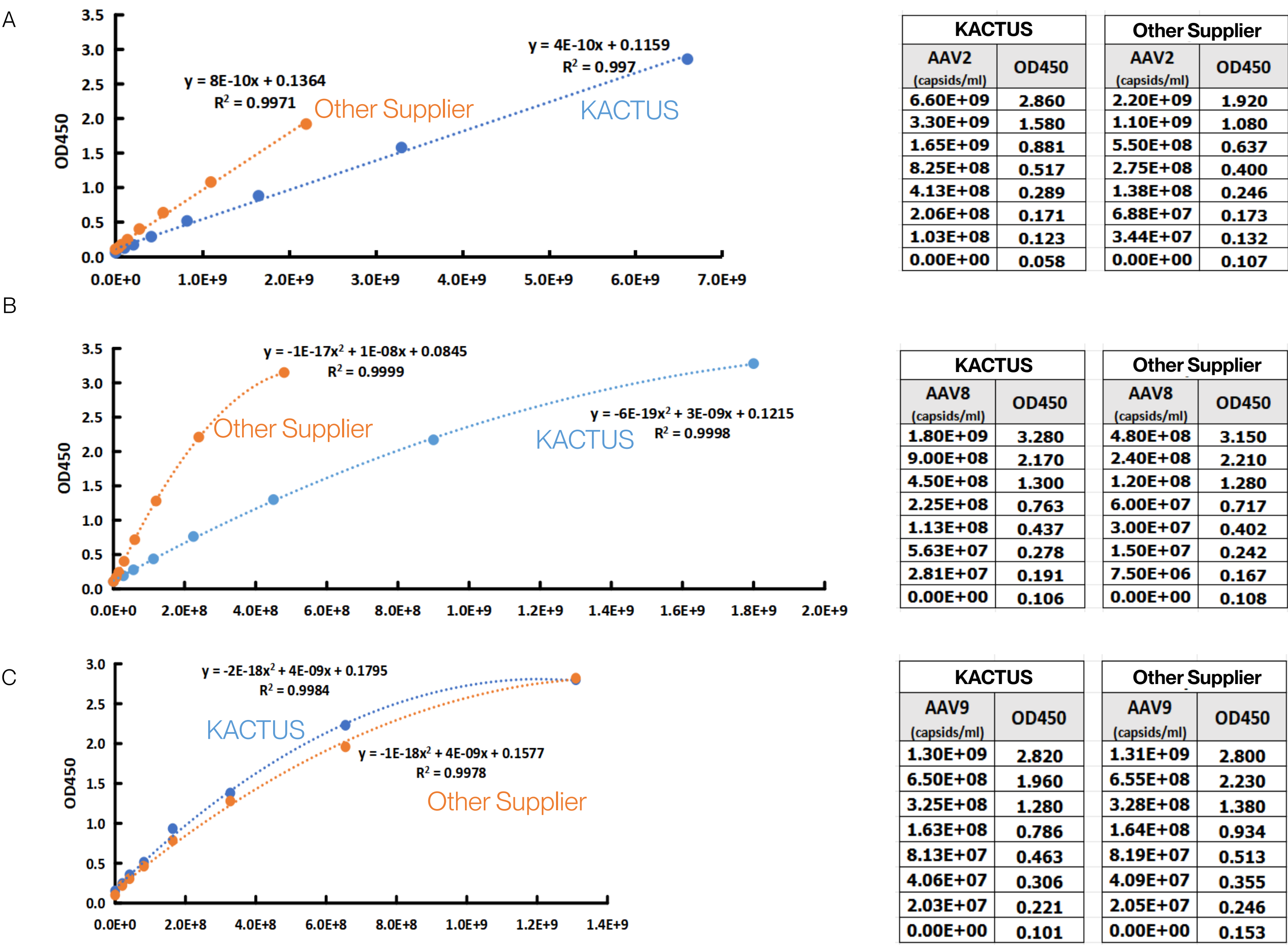


Figure 3. Comparison of the linear range and signal-to-noise ratio of the AAV Titration ELISA Kits produced from KACTUS and another supplier. Examples of KACTUS AAV2 (A), AAV8 (B) and AAV9 (C) ELISA kits were shown to demonstrate a wider linear range and higher signal-to-noise ratio, suggesting a lower background and better detection range.

High Intra-Assay Precision & Recovery Rate

In addition to linear range, intra-assay precision, and kit recovery rate are critical indicators of kit quality. Intra-assay precision (also known as repeatability) measures the variability among data points within a single assay. Typically, a coefficient of variation (CV) below 10% is desirable. The kit recovery rate assesses the impact of the diluent or matrix used in the assay on quantification. Generally, a recovery rate ranging from 80% to 120% indicates minimal interference from the diluent or matrix. We demonstrate good intra-assay precision and kit recovery rate using our AAV5 ELISA Kit as an example.

AAV5 10 tests	1.00E+09		2.50E+08		6.25E+07	
	OD450	AAV5 (capsids/ml)	OD450	AAV5 (capsids/ml)	OD450	AAV5 (capsids/ml)
1	2.160	8.59E+08	0.904	2.87E+08	0.313	7.21E+07
2	2.110	8.31E+08	0.854	2.68E+08	0.301	6.80E+07
3	2.150	8.53E+08	0.894	2.83E+08	0.316	7.31E+07
4	2.130	8.42E+08	0.897	2.84E+08	0.317	7.35E+07
5	2.130	8.42E+08	0.915	2.91E+08	0.319	7.42E+07
6	2.090	8.20E+08	0.895	2.84E+08	0.310	7.11E+07
7	2.130	8.42E+08	0.891	2.82E+08	0.311	7.14E+07
8	2.150	8.53E+08	0.896	2.84E+08	0.314	7.25E+07
9	2.060	8.04E+08	0.877	2.77E+08	0.314	7.25E+07
10	2.100	8.26E+08	0.861	2.71E+08	0.298	6.69E+07
Average (AV)	2.1210	8.37E+08	0.8884	2.81E+08	0.3113	7.15E+07
Standard Deviation (SD)	0.0311	1.70E+07	0.0189	7.09E+06	0.0068	2.35E+06
coefficient of variation (CV)	1.47%	2.03%	2.13%	2.52%	2.18%	3.29%

Table 2. Assessment of intra-assay precision and recovery rate with the AAV5 Titration Kit. 10 measurements were conducted for each of 3 distinct samples. Standard deviations were calculated, along with their corresponding coefficients of variation (CV). An acceptable CV threshold is set at less than 10%.

Stability Testing: Effective Even After 7 Days at 37°C

We performed the stability study against AAV Titration ELISA Kits at 37°C for 7 days. The results revealed that the standard curve maintained a good fit and linearity at different time points, demonstrating the kit's outstanding reagent stability (Fig. 5).

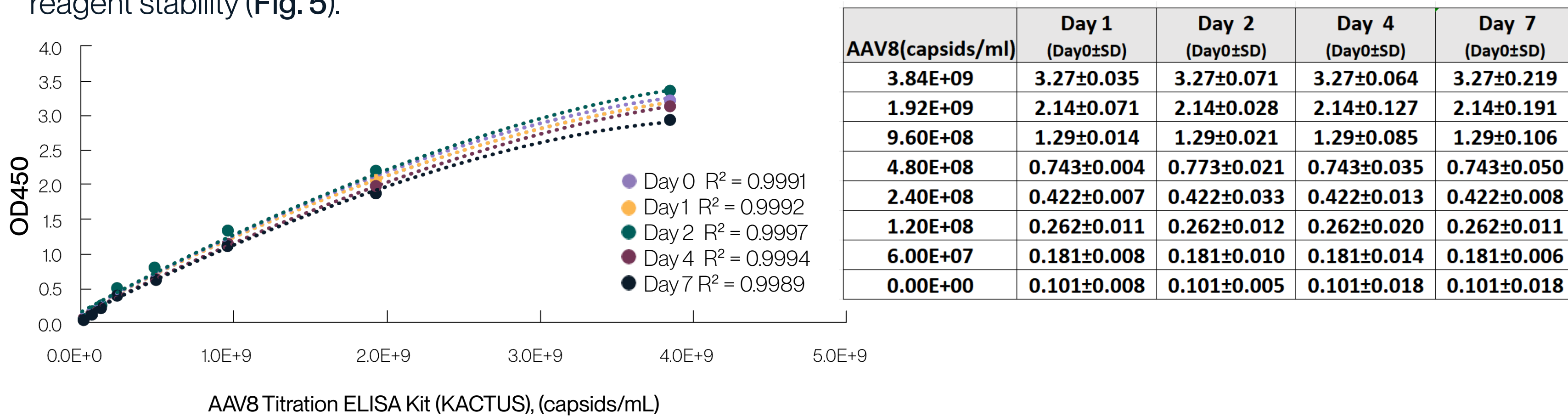


Figure 5. Stability testing for AAV8 Titration ELISA Kit. The kit components were stored at 37°C for 7 days. A standard curve was run on day 1, 2, 4, and 7.

Conclusion

KACTUS has successfully developed high-quality AAV Titration ELISA Kits against canonical serotypes, known for their precision, consistent performance, and excellent recovery rates to guarantee accurate AAV titration, which is crucial for AAV-based gene therapy development.

Our R&D team's strong expertise in AAV biology and assay development make feasible custom AAV ELISA kit development. KACTUS provides high-quality solutions for gene therapy companies to develop custom kits optimized for their specific AAV variants, ensuring accurate and reliable results.

References
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