Tailored AAV Solutions and Customized Variant Kit Development

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Introduction

Adeno-associated viruses (AAVs) have been a potent tool for gene delivery. Different 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 clinical use. The enzyme-linked immunosorbent assay (ELISA) is considered to be a simple and accurate method for quantifying AAV viral particle titers. However, it is challenging to generate high-quality monoclonal antibodies specific to AAV serotypes due to the weak immunogenicity of AAVs.

As a result, KACTUS relies on our self-built platform to independently screen and produce monoclonal antibodies specific to AAV serotypes. We have developed a series of highly-specific AAV titration ELISA kits, that deliver performance on par with, if not superior to, that of leading industry standards. This platform can also support the customization of other serotype-specific AAV titration ELISA kits, to meet the demands for quantifying AAV drug viral capsids.

Background: AAV Structure

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. See **Figure 1.** Most of these epitopes overlap with receptor-binding sites on the capsid, making them the primary target for AAV variant engineering.

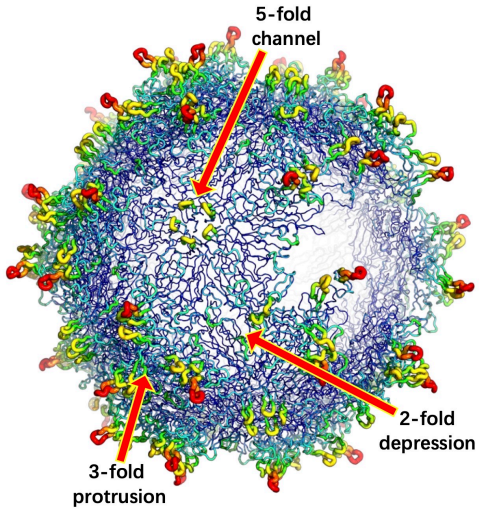


Figure 1. The overall structural characteristics of the AAV particle.

AAV Titration ELISA Kit Development

Monoclonal antibodies (MAbs) used in titration kits must recognize conformational epitopes on intact particles without binding to the individual VP subunits. The development of high-quality titration kits is made possible by the broad linear range and high specificity of the KACTUS Mab to AAV serotype. We show that KACTUS AAV2, 5, 6, 8, and 9 titration ELISA kits are of equivalent or superior quality to popular AAV titration kits on the market. See **Table 1.**

AAV serotype	AAV Standard range [capsids/mL]	Titration kit Sensitivity [capsids/mL]
AAV2	6.59E+09 – 1.03E+08	5E+07
AAV5	4.00E+09 – 6.25E+07	3E+07
AAV6	2.50E+09 – 3.91E+07	5E+07
AAV8	1.80E+09 – 2.81E+07	2E+07
AAV9	1.30E+09 – 2.03E+07	1E+07

Table 1. Linear range of sensitivity of KACTUS AAV titration kits.

Overcoming weak AAV immunogenicity with KACTUS' proprietary immunization scheme

AAV2 exhibits the lowest immunogenicity compared to other AAV serotypes, making it challenging to obtain high-quality monoclonal antibodies (MAb) targeting AAV2. Therefore, we have developed a distinctive AAV immunization approach to enhance AAV2 anti-serum titers, thereby stimulating robust antibody responses. KACTUS' proprietary immunization scheme overcomes the weak AAV immunogenicity for supporting high-quality anti-AAV monoclonal antibody screening. See **Figure 2**.

The method maintains a remarkable level of consistency as the serum titers within the same group of mice consistently align. Across all AAV instances, our technique consistently elevates AAV anti-serum titers, resulting in the production of high-performance monoclonal antibodies.

Figure 2. Comparison of anti-AAV2 serum titers for KACTUS' proprietary versus standard immunization protocols.

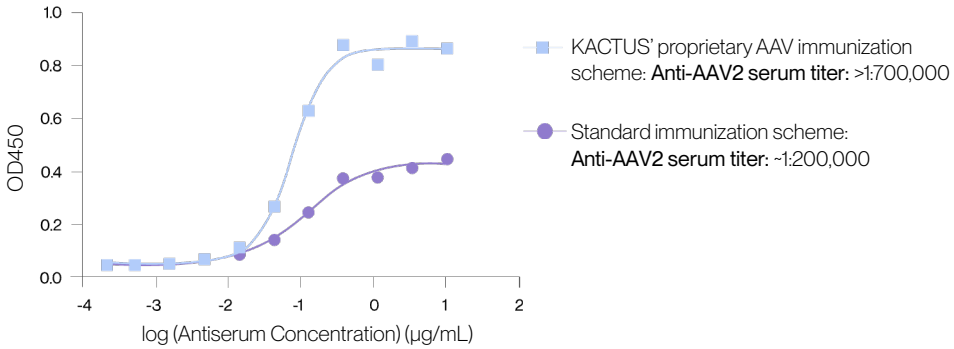
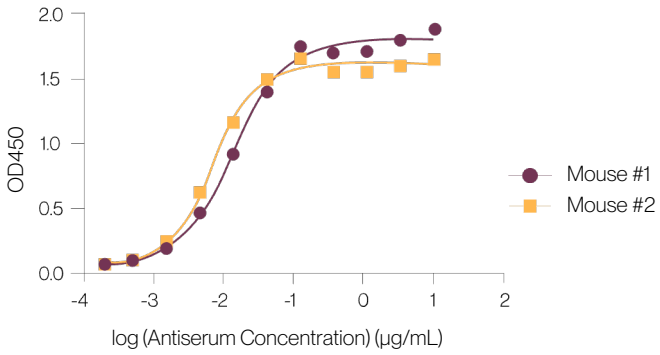


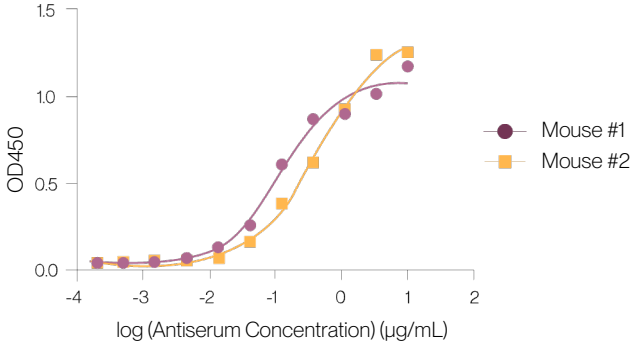
Figure 3. KACTUS' proprietary AAV immunization scheme enables high titer of anti-AAV5 (Figure 3A), anti-AAV8 (Figure 3B), and anti-AAV9 (Figure 3C) antibodies.

Figure 3A.



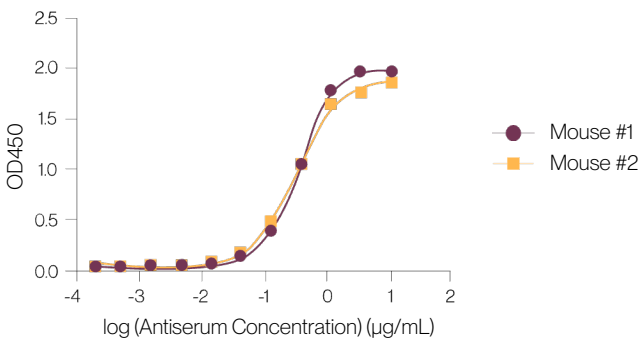
Anti-AAV5 serum titer: >1:700,000

Figure 3B.



Anti-AAV8 serum titer: >1:200,000

Figure 3C.



Anti-AAV9 serum titer: >1:200,000

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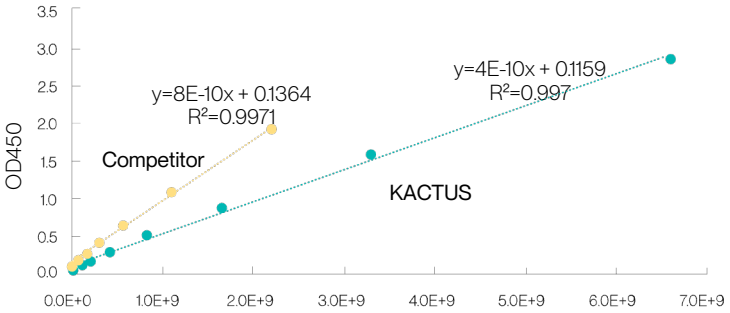
Comparing the linear range of KACTUS AAV Titration ELISA kit to another top industry supplier

To ensure accurate quantification, minimizing the need for extensive sample dilution is essential to have a broad linear range in AAV Titration ELISA kits. Since the characteristics of the MAbs directly impact the kit's detection span, greater MAb sensitivity results in a more limited linear range. Consequently, we selected MAbs with extended detection ranges during the antibody screening process for the development of the KACTUS AAV titration ELISA kit.

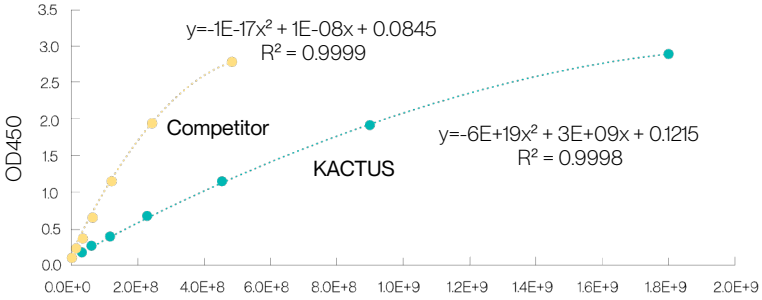
Figure 4A. AAV2 Titration. KACTUS vs. a leading AAV ELISA Kit. KACTUS AAV2 Titration ELISA kit has a broader quantification linear range and a better signal-to-noise ratio than the competitor kit.



KACTUS	
AAV2 (capsids/mL)	OD450
6.60E+09	2.860
3.30E+09	1.580
1.65E+09	0.881
8.25E+08	0.517
4.13E+08	0.289
2.06E+08	0.174
1.03E+08	0.123
0.00E+00	0.058

Competitor	
AAV2 (capsids/mL)	OD450
2.20E+09	1.920
1.10E+09	1.080
5.50E+09	0.637
2.75E+08	0.400
1.38E+08	0.246
6.88E+08	0.173
3.44E+08	0.132
0.00E+00	0.107

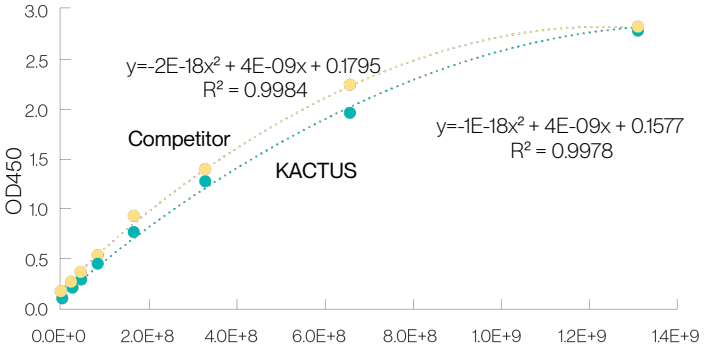
Figure 4B. AAV8 Titration. When compared to the competitor kit, the quantification linear range of the KACTUS AAV8 Titration ELISA kit is notably broader.



KACTUS	
AAV8 (capsids/mL)	OD450
1.80E+09	3.280
9.00E+08	2.170
4.50E+08	1.300
2.25E+08	0.763
1.13E+08	0.437
5.63E+07	0.278
2.81E+07	0.191
0.00E+00	0.106

Competitor	
AAV8 (capsids/mL)	OD450
4.80E+08	3.150
2.40E+08	2.210
1.20E+08	1.280
6.00E+07	0.717
3.00E+07	0.402
1.50E+07	0.242
7.50E+06	0.167
0.00E+00	0.108

Figure 4C. AAV9 Titration. The KACTUS AAV9 ELISA kit has a lower background and higher signal-to-noise ratio, with a comparable linear range to a leading competitor.



KACTUS	
AAV9 (capsids/mL)	OD450
1.30E+09	2.820
6.50E+08	1.960
3.25E+08	1.280
1.63E+08	0.786
8.13E+07	0.463
4.06E+07	0.306
2.03E+07	0.221
0.00E+00	0.101

Competitor	
AAV9 (capsids/mL)	OD450
1.31E+09	2.800
6.55E+08	2.230
3.28E+08	1.380
1.64E+08	0.934
8.19E+07	0.513
4.09E+07	0.355
2.05E+07	0.246
0.00E+00	0.153

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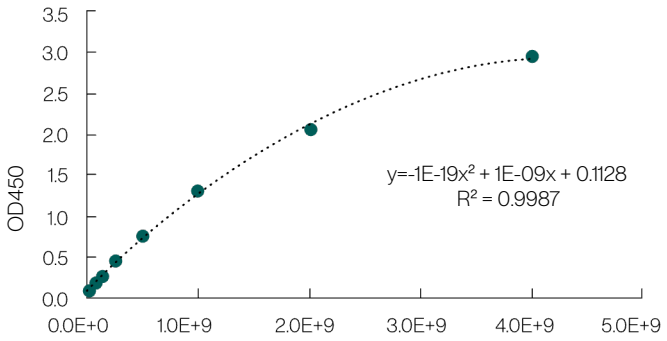
Linear ranges and-standard-curves-of AAV5 and AAV6 titration ELISA kits

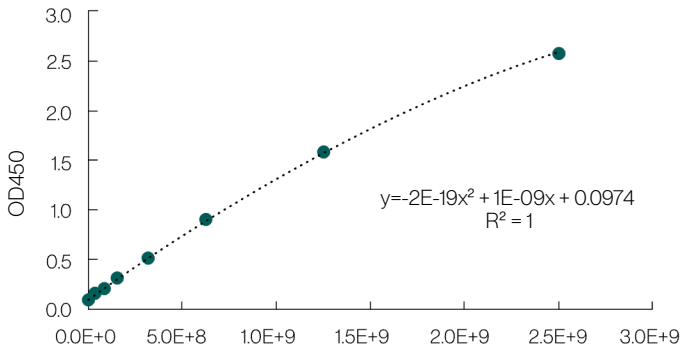
Figure 5A. Standard curves of AAV5 Titration ELISA Kit



AAV5 Titration ELISA Kit (KACTUS), (capsids/mL)

AAV5 (capsids/mL)	OD450
4.00E+09	2.960
2.00E+09	2.050
1.00E+09	1.310
5.00E+08	0.749
2.50E+08	0.451
1.25E+08	0.263
6.25E+07	0.174
0.00E+00	0.077

Figure 5B. Standard curves of AAV6 Titration ELISA Kit.

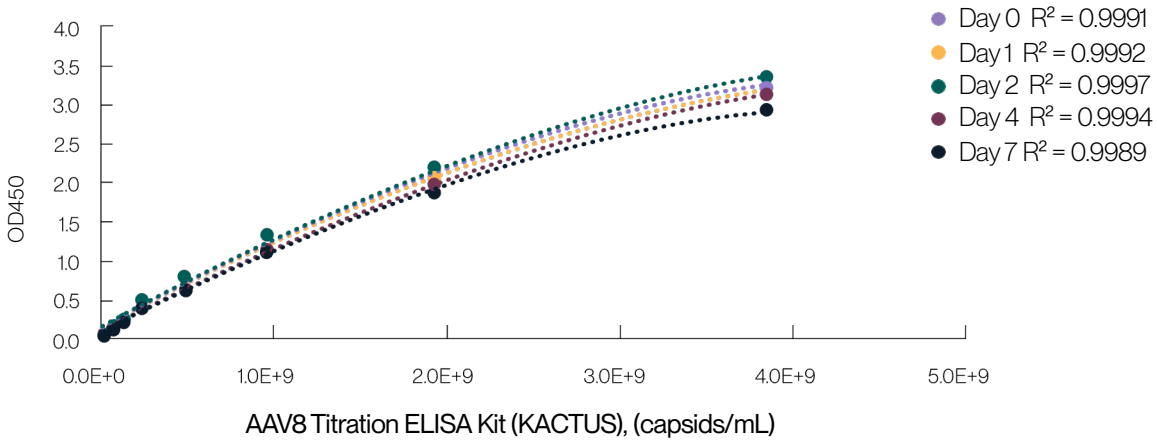


AAV6 Titration ELISA Kit (KACTUS), (capsids/mL)

AAV6 (capsids/mL)	OD450
2.50E+09	2.580
1.25E+09	1.590
6.25E+08	0.908
3.13E+08	0.521
1.56E+08	0.319
7.81E+07	0.208
3.91E+07	0.156
0.00E+00	0.085

KACTUS AAV titration ELISA kits offer good stability

Figure 6. AAV Titration ELISA kit stability study. KACTUS AAV8 Titration ELISA Kit components were stored at 37°C for up to 7 days. A standard curve for the kit was run on days 0, 1, 2, 4, and 7. Results are shown below and show $R^2 > 0.99$ for all standard curves.



AAV8 (capsids/ml)	Day 1 (Day 0 ± SD)	Day 2 (Day 0 ± SD)	Day 4 (Day 0 ± SD)	Day 7 (Day 0 ± SD)
3.84E+09	3.27 ± 0.035	3.27 ± 0.071	3.27 ± 0.064	3.27 ± 0.219
1.92E+09	2.14 ± 0.071	2.14 ± 0.028	2.14 ± 0.127	2.14 ± 0.191
9.60E+08	1.29 ± 0.014	1.29 ± 0.021	1.29 ± 0.085	1.29 ± 0.106
4.80E+08	0.743 ± 0.004	0.773 ± 0.021	0.743 ± 0.035	0.743 ± 0.050
2.40E+08	0.422 ± 0.007	0.422 ± 0.033	0.422 ± 0.013	0.422 ± 0.008
1.20E+08	0.262 ± 0.011	0.262 ± 0.012	0.262 ± 0.020	0.262 ± 0.011
6.00E+07	0.181 ± 0.008	0.181 ± 0.010	0.181 ± 0.014	0.181 ± 0.006
0.00E+00	0.101 ± 0.008	0.101 ± 0.005	0.101 ± 0.018	0.101 ± 0.018

Table 5. AAV titration kit stability test. Our AAV8 ELISA Titration kit components were stored at 37°C for up to 7 days. A standard curve for the kit was run on days 0, 1, 2, 4, and 7. Results shown below and show $R^2 > 0.99$ for all standard curves.

AAV titration ELISA kits offer high intra-assay precision and good recovery rate

Apart from assessing linear range and kit stability, it is essential to consider intra-assay precision, inter-assay precision, and kit recovery rate as vital 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. Inter-assay precision reflects the reproducibility of results across assays conducted on different days. Achieving an inter-assay precision with a CV below 10% ensures consistent results over time and across various kits.

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.

Test Number	Samples of various AAV5 capsid concentrations (capsids/mL)					
	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	8.31+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	0314	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	1.170E+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 with the AAV5 Titration ELISA Kit. To evaluate the intra-assay precision of the AAV5 titration ELISA ELISA, 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%.

Summary

Our proprietary mouse immunization scheme has been proven to be effective in overcoming weak AAV immunogenicity and gaining high anti-serum titers. Mouse monoclonal antibodies that only recognize intact AAV particles with high specificity were used for making AAV titration Elisa kits. Among our collection of native AAV2, 5, 6, 8, and 9 titration ELISA kits, some are comparable to well-known competitor kits and some have a wider linear range than industry standards in order to better meet customers needs. Looking ahead, with the increasing application of engineered and novel AAV vectors in gene therapy, KACTUS is prepared to offer customized anti-AAV antibody discovery and tailored development of specialized titration kits.

Product List

Catalog #	Product Name
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

**Launching Q1 2024.*

KACTUS

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Version 18-10-2024