Product Instruction Manual-TrueX®-AAV Titration Kit (ELISA)

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Introduction

The GeneMedi AAV Titration ELISA Kit is an Enzyme-linked immunosorbent assay (ELISA) designed for the quantitative determination of multiple adeno-associated virus (AAV) serotype particles in cell culture supernatants and purified virus preparations. GeneMedi offers AAV2/5/6/8/9 Serotype Specific Titration Kit (ELISA).

Accurate titration of AAV particles is a critical quality control step in gene therapy research and application. This kit enables precise detection and can also quantify novel AAV vectors created through mutations, DNA-shuffling, or site-specific insertions.

Product list

Cat No	Product Name	Target Serotype(s)
GMV-TrueX-ELISA-2-1-96	TrueX®-AAV2 Serotype Titration Kit (ELISA)	AAV2
GMV-TrueX-ELISA-5-1-96	TrueX®-AAV5 Serotype Titration Kit (ELISA)	AAV5
GMV-TrueX-ELISA-6-1-96	TrueX®- AAV6 Serotype Titration Kit (ELISA)	AAV6
GMV-TrueX-ELISA-8-1-96	TrueX®- AAV8 Serotype Titration Kit (ELISA)	AAV8
GMV-TrueX-ELISA-9-1-96	TrueX®-AAV9 Serotype Titration Kit (ELISA)	AAV9

Kit components & Storage

Components	Size	Format	Storage
MTP (Microtiter Plate)	12 x 8-well strips	Vacuum packing with desiccant bag	2-8℃
Assay Buffer (10x)	50 mL	Tube, dilute before use	2-8℃
Biotin conjugated anti-AAV antibody (100x)	150 µL	Tube, dilute before use	2-8℃
HRP-SA (HRP labelled streptavidin, 1000x)	15 µL	Tube, dilute before use	2-8℃
TMB	15 mL	Tube, ready to use	2-8℃
Stop Solution	15 mL	Tube, ready to use	2-8℃

Notes:

- This kit is intended for **research use only**.
- Do not use the kit if the packaging seal is broken upon arrival.
- If there are any remaining microtiter strips after opening, immediately reseal them in the foil pouch with the desiccant bag and store at 2-8°C.

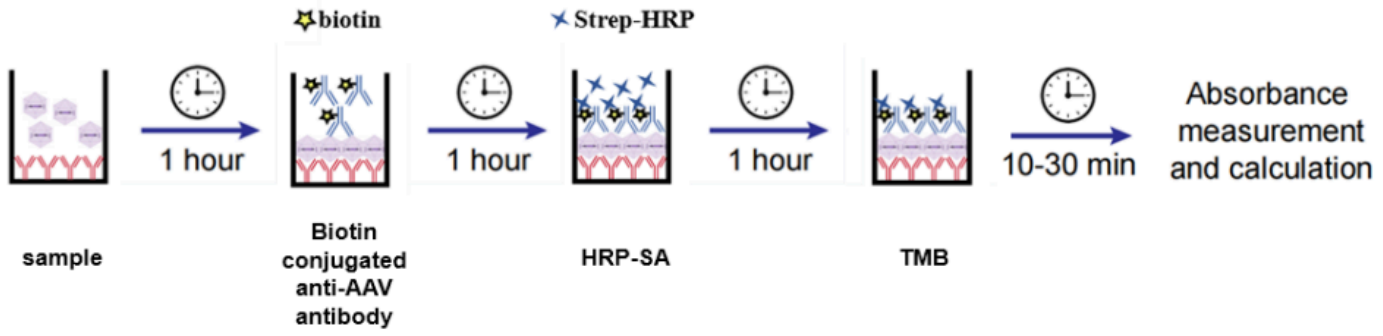
Shelf Life: At least 6 months (stability confirmed through 6 months of testing; longer-term stability is currently under assessment).

Principle of Testing

This ELISA kit utilizes a proprietary antibody pair developed by GeneMedi, featuring broad AAV serotype-binding capabilities.

1. **Capture:** The microtiter plate is pre-coated with a capture antibody. Test samples and standards are added directly to the wells, allowing the AAV particles to bind to the plate.
2. **Detection:** After incubation and washing, a biotin-conjugated anti-AAV secondary antibody is added to the wells to bind the captured AAV particles.
3. **Signal Generation:** Following another wash step, HRP-conjugated streptavidin is introduced. Finally, TMB substrate is added, producing a measurable colorimetric signal. The total assay time is approximately 3.5 hours.

Short protocol Flowchart



Titration procedure

A. Kit Preparation

1. General Reagent Preparation

- Bring the MTP well strips and the 10x Assay Buffer to room temperature before beginning the procedure.
- Prepare the 1x Assay Buffer by diluting the 10x Assay Buffer with distilled water (ddH2O).
- Note: All AAV standards and test samples must be diluted using 1x PBS (pH 7.4).

2. AAV Standard Reconstitution

The AAV standards provided in this kit are lyophilized. Reconstitute each standard vial with the appropriate volume of distilled water (or 1x PBS, as designated by the lot-specific QC sheet) to achieve the **Stock Titer** listed in Table 1 below.

Table 1: AAV Standard Specifications & Reconstitution

Catalog No.	Serotype	Reconstituted Volume	Stock Titer (capsids/mL)	Top Standard Working Titer (capsids/mL)	Storage (Post-Reconstitution)
GMV-TrueX-ELISA-2-1-96	AAV2	40 µL * 4	1.0E+11	5.0E+09	-80°C
GMV-TrueX-ELISA-5-1-96	AAV5	40 µL * 4	1.0E+12	5.0E+10	-80°C
GMV-TrueX-ELISA-6-1-96	AAV6	40 µL * 4	1.0E+11	5.0E+09	-80°C
GMV-TrueX-ELISA-8-1-96	AAV8	40 µL * 4	1.0E+11	5.0E+09	-80°C
GMV-TrueX-ELISA-9-1-96	AAV9	40 µL * 4	1.0E+11	5.0E+09	-80°C

B. Standard and Specimen Dilution

We recommend diluting the reconstituted AAV Standards in 1x PBS (pH 7.4) using 1:2 serial dilution steps.

Important: The optimal detection range and starting concentration differ based on the AAV serotype being tested.

- **For AAV2, AAV6, AAV8, and AAV9:** The top standard working titer is **5.0E+09 capsids/mL**. First, dilute the Stock Titer (1.0E+11 capsids/mL) 1:20 in 1x PBS to create standard **Std 1** .
- **For AAV5:** The top standard working titer is **5.0E+10 capsids/mL**. First, dilute the Stock Titer (1.0E+12 capsids/mL) 1:20 in 1x PBS to create standard **Std 1** .

Standard Curve Preparation Guide:

Dilution Step	Preparation (for all serotypes)	Final Concentration (AAV2, 6, 8, 9)	Final Concentration (AAV5)
Std 1 (Top)	Dilute reconstituted Stock 1:20 in 1x PBS	5.00E+09 capsids/mL	5.00E+10 capsids/mL
Std 2 (1:2)	250 µL Std 1 + 250 µL 1x PBS	2.50E+09 capsids/mL	2.50E+10 capsids/mL
Std 3 (1:4)	250 µL Std 2 + 250 µL 1x PBS	1.25E+09 capsids/mL	1.25E+10 capsids/mL
Std 4 (1:8)	250 µL Std 3 + 250 µL 1x PBS	6.25E+08 capsids/mL	6.25E+09 capsids/mL
Std 5 (1:16)	250 µL Std 4 + 250 µL 1x PBS	3.125E+08 capsids/mL	3.125E+09 capsids/mL
Std 6 (1:32)	250 µL Std 5 + 250 µL 1x PBS	1.56E+08 capsids/mL	1.56E+09 capsids/mL
Std 7 (1:64)	250 µL Std 6 + 250 µL 1x PBS	7.80E+07 capsids/mL	7.80E+08 capsids/mL
Blank	1x PBS only (0 capsids/mL)	0 capsids/mL	0 capsids/mL

Pre-dilute your unknown specimens containing AAV particles in 1x PBS (pH 7.4) in serial dilution steps to fall within the optimal detection range of the ELISA curve (approx. 2.5E+09 to 3.0E+08 capsids/mL for AAV2/6/8/9, or 10-fold higher for AAV5). Multiple dilutions may be required for accurate data acquisition.

C. Example Microtiter Plate Layout

We recommend running all standards and samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Sp 1	Sp 1	Sp 9	Sp 9						
B	Std 2	Std 2	Sp 2	Sp 2	Sp 10	Sp 10						
C	Std 3	Std 3	Sp 3	Sp 3	etc.	etc.						
D	Std 4	Std 4	Sp 4	Sp 4								
E	Std 5	Std 5	Sp 5	Sp 5								
F	Std 6	Std 6	Sp 6	Sp 6								
G	Std 7	Std 7	Sp 7	Sp 7								
H	Blank	Blank	Sp 8	Sp 8								

(Sp = Specimen/Sample Dilution)

D. ELISA Assay procedure

1. **Pre-wet Wells:** Add 200 µL of 1x Assay Buffer to each well. Incubate for 5 minutes at room temperature, then discard the liquid.
2. **Sample Addition:** Add 100 µL of prepared standards and pre-diluted test samples into their designated wells.
3. **Incubation:** Seal the plate and incubate at 37°C for 1 hour.
4. **Wash Step:** Discard the liquid and wash each well with 200 µL of 1x Assay Buffer. Repeat this step for a total of **three** washes. Tap the plate firmly on clean paper towels to remove residual buffer.
5. **Secondary Antibody:** Dilute the 100x biotin-conjugated secondary antibody to a 1x working concentration using 1x Assay Buffer. Add 100 µL of the diluted biotin-conjugated antibody to each well.
6. **Incubation:** Seal the plate and incubate at 37°C for 1 hour.
7. **Wash Step:** Discard the liquid and wash each well with 200 µL of 1x Assay Buffer for a total of **three** washes.
8. **HRP Detection:** Dilute the 1000x HRP-SA solution to a 1x working concentration using 1x Assay Buffer. Add 100 µL of the diluted HRP-SA solution to each well.
9. **Incubation:** Seal the plate and incubate at 37°C for 1 hour.
10. **Wash Step:** Discard the liquid and wash each well with 200 µL of 1x Assay Buffer for a total of **three** washes.
11. **Substrate Development:** Add 100 µL of TMB Substrate into each well. Incubate at 37°C in the dark for 5–30 minutes. *(Note: Monitor the color development closely; check the reaction every 3 minutes initially and stop the reaction at the appropriate time).*
12. **Stop Reaction:** Add 100 µL of Stop Solution into each well to terminate the reaction. The color will change from blue to yellow.
13. **Measurement:** Immediately measure the absorbance at 450 nm using a microplate spectrophotometer.

Calculation of Results

1. Calculate the mean absorbance (OD450) for each duplicate standard, control, and sample.
2. Subtract the average OD value of the Blank (zero standard) from all other mean OD values to obtain the corrected absorbance.
3. Create a standard curve by plotting the known standard concentrations (capsids/mL) on the Y-axis against their corrected absorbance values on the X-axis.
4. Use a two-parameter logistic model to fit the curve.
5. Determine the sample concentrations by interpolating the OD values against the standard curve.
6. Multiply the interpolated value by the sample's corresponding dilution factor to obtain the final capsid titer (capsids/mL) of the original specimen.

(Note: The detection range for unknown AAV samples must fall within the linear portion of the generated AAV standard curve. Samples exceeding the highest standard must be further diluted and reassayed).

Example data

Standard capsids/ml	OD450
5.00E+09	1.3131
2.50E+09	0.654
1.25E+09	0.3317
6.25E+08	0.1996
3.13E+08	0.0968
1.56E+08	0.0431
7.81E+07	0.0199
0 (PBS)	0

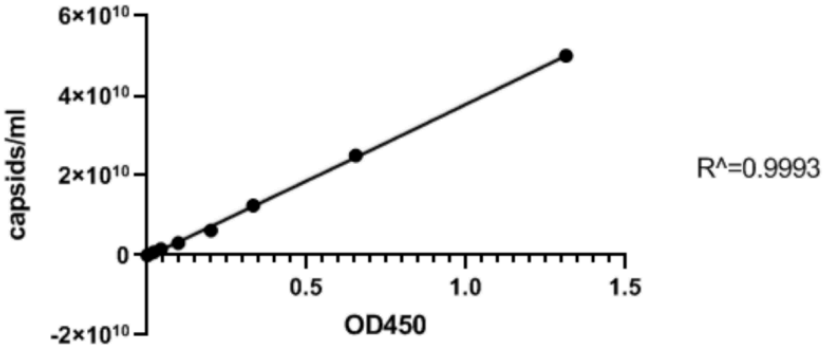


Figure 1. AAV2 capsid titration curve generated using the GenMedi AAV Titration ELISA kit (GMV-TrueX-ELISA-2-1-96).

The table shows the standard concentrations of AAV 2 capsids (capsids/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation (R^2 = 0.9998) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard capsids/ml	OD450
5.00E+10	1.3415
2.50E+10	0.7329
1.25E+10	0.4079
6.25E+09	0.2363
3.13E+09	0.1323
1.56E+09	0.0644
7.81E+08	0.0357
0	0

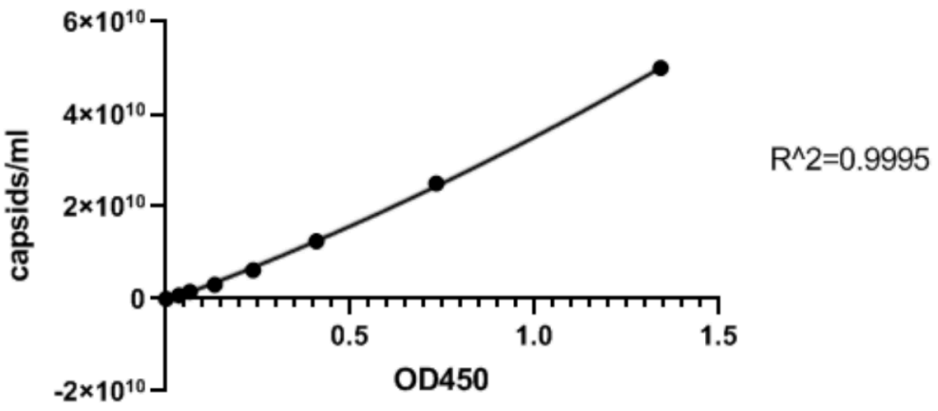


Figure 2. AAV5 capsid titration curve generated using the GenMedi AAV Titration ELISA kit (GMV-TrueX-ELISA-5-1-96).

The table shows the standard concentrations of AAV5 capsids (capsids/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation (R^2 = 0.9995) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard capsids/ml	OD450
5.00E+09	1.5166
2.50E+09	0.8054
1.25E+09	0.4156
6.25E+08	0.1997
3.13E+08	0.0935
1.56E+08	0.0483
7.81E+07	0.0201
0	0.0000

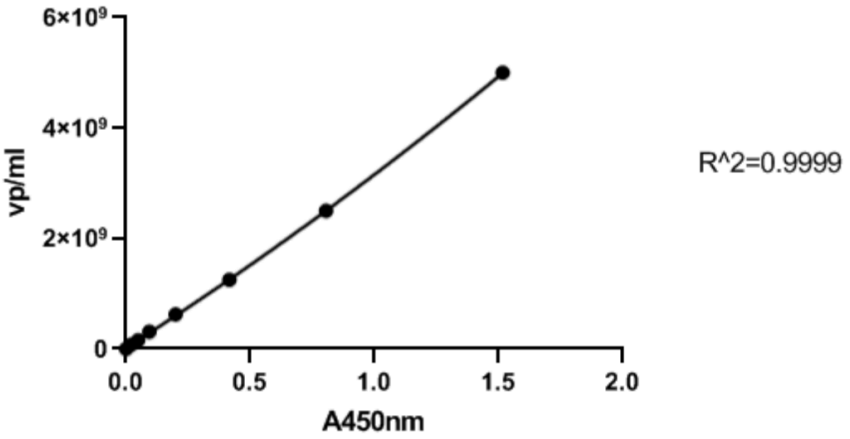


Figure 3. AAV6 capsid titration curve generated using the GenMedi AAV Titration ELISA kit (GMV-TrueX-ELISA-6-1-96).

The table shows the standard concentrations of AAV 6 capsids (capsids/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation (R^2 = 0.9998) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard capsids/ml	OD450
5.00E+09	1.1155
2.50E+09	0.5449
1.25E+09	0.2726
6.25E+08	0.1446
3.13E+08	0.0719
1.56E+08	0.0336
7.81E+07	0.0308
0	0.0000

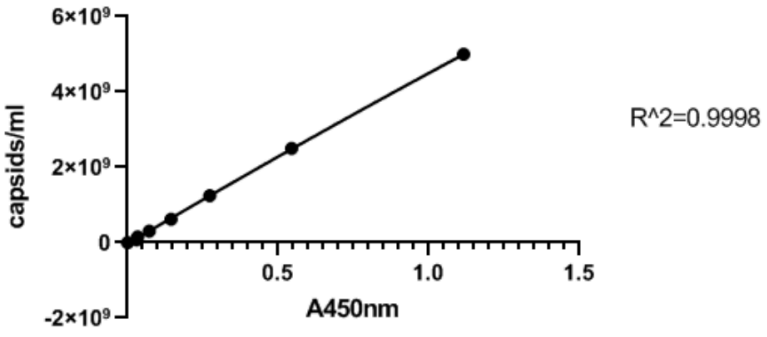


Figure 4. AAV8 capsid titration curve generated using the GenMedi AAV Titration ELISA kit (GMV-TrueX-ELISA-8-1-96).

The table shows the standard concentrations of AAV 8 capsids (capsids/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation (R^2 = 0.9998) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard capsids/ml	OD450
5.00E+09	2.3692
2.50E+09	1.5129
1.25E+09	0.7212
6.25E+08	0.3888
3.13E+08	0.1978
1.56E+08	0.1024
7.81E+07	0.0559
0	0.0140

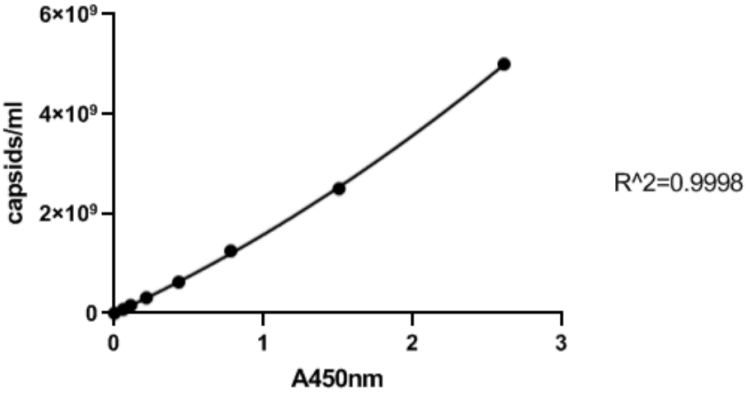


Figure 5. AAV9 capsid titration curve generated using the GenMedi AAV Titration ELISA kit (GMV-TrueX-ELISA-9-1-96).

The table shows the standard concentrations of AAV 9 capsids (capsids/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation (R^2 = 0.9998) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.