AAV Titration ELISA kit (universal)

Product Instruction Manual

The GeneMedi AAV Titration ELISA Kit is an Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of multiple adeno-associated virus (AAV) serotype particles in cell culture supernatants and purified virus preparations.

Catalog No.	**GMV-TrueX-ELISA-U-1-96-1P**
Contents	**12 x 8 Determinations**
Storage	**2- 8 ° C**

Kit components

Components	Size	Format	Storage
MTP (Microtiter Plate)	12 x 8-well strips	Vacuum packing with desiccant bag	2-8°C
Assay Buffer (20x)	50 mL	Tube, dilute before use	2-8°C
Biotin conjugated anti-AAV antibody (370x)	50 µL	Tube, dilute before use	2-8°C
HRP-SA (HRP labelled streptavidin, 5000x)	5 µL	Tube, dilute before use	2-8°C
TMB	15 mL	Tube, ready to use	2-8°C
Stop Solution	15 mL	Tube, ready to use	2-8°C

Note:

This kit is intended for research use only. Do not use the kit if the seal is broken. If there are any remaining strips after opening, reseal them with the desiccant bag.

Shelf Life:

At least 6 months (stability confirmed through 6 months of testing; longer-term stability is still being assessed).

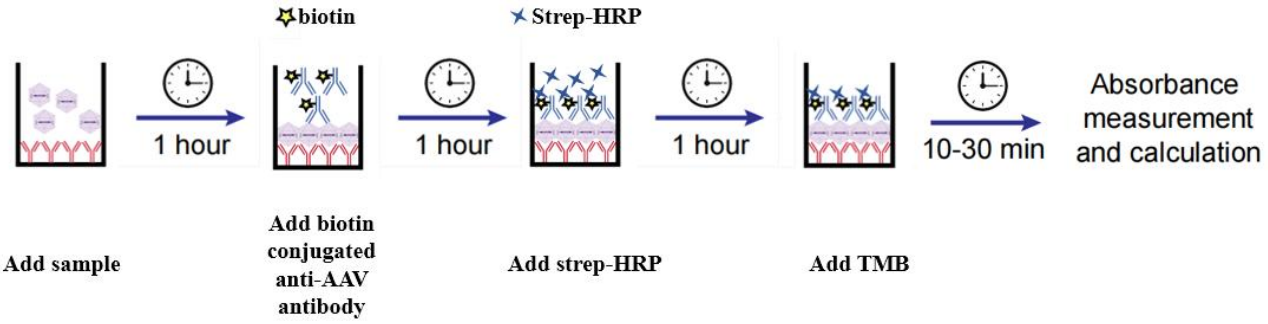
Introduction

The adeno-associated virus (AAV) is a small, non-enveloped virus, approximately 25 nm in size, widely used in research and gene therapy.

Accurate titration of AAV particles is a critical quality control step in their research and application. GeneMedi AAV Titration ELISA kit enables the detection of multiple AAV serotypes, including AAV2, AAV5, AAV6, AAV8, and AAV9. It can be also quantify novel AAV vectors created through mutations, DNA-shuffling, or point-specific insertion.

Principle of Testing

The ELISA kit is utilizes an antibody pair with broad AAV serotype-binding capability, developed by GeneMedi. The microtitter plate is pre-coated with a capture antibody. Test samples are added directly to the plate for AAV capture. After incubation and washing, a biotin-conjugated anti-AAV antibody is added, followed by incubation and washing. Finally, HRP-conjugated streptavidin is added for detection. The total assay time is approximately 3.5 hours.



Titration procedure

A. Kit preparation

- Bring the wellstrips and Assay Buffer (20x) to room temperature;
- Prepare the 1x assay buffer by diluting the 20x Assay Buffer with distilled water (ddH2O).
- Dilute the AAV standard sample with 1x PBS (pH7.4) to the following concentrations:5e9 vp/mL,
- 2.5e9 vp/mL, 1.25e9 vp/mL, 6.25e8 vp/mL, 3.125e8 vp/mL, 1.56e8 vp/mL, 7.8e7 vp/mL.

Note:

1. *All AAV standards and samples must be diluted with 1*PBS (pH 7.4)*

B. ELISA procedure

a. Add 200 µL of 1x Assay Buffer to each well, incubate for 5 minutes at room temperature, and discard the supernatant.
b. Add 100 µL of standards and samples into each well;

Note:

1. *The optimal detection range is approximately 2.5e9 vp/mL to 3e8 vp/mL.depending on the AAV serotype.*
2. *Multiple dilutions may be required to obtain accurate data.*

c. Incubate at 37 °C for 1 hour.
d. Discard the supernatant and wash each well with 200uL of 1x Assay Buffer for three times.
e. Dilute the biotin-conjugated secondary antibody with 1x Assay Buffer.
f. Add 100 µL of the diluted biotin-conjugated antibody and incubate for 1 hour at 37 °C.
g. Discard the supernatant and wash each well with 200uL of 1x Assay Buffer for three times;

h. Dilute the HRP-detection solution with 1x Assay Buffer.
i. Add 100 µL of the diluted HRP-detection solution and incubate for 1 hour at 37 °C.
j. Discard the supernatant and wash each well with 200uL of 1x Assay Buffer for three times;
k. Add 100 µL of TMB into each well, incubate at 37 °C for 5-30min;
Note:*Check the reaction every 3 minutes initially and stop the reaction at the appropriater time.*
l. Add 100 µl of Stop Solution into each well;
m. Measure the absorbance at 450 nm using a microplate spectrophotometer at 450 nm (optional: reference wavelength at 650 nm).

C. Calculation

a. Calculate the mean absorbance for each standard, control, and sample. Subtract the average optical density (OD) of the zero standard from all values;
b. Create a standard curve by plotting standard concentrations on the y-axis and corrected absorbance values on the x-axis. Use a two-parameter logistic model to fit the curve and determine sample concentrations;
c. Detection range. The detection range for AAV particles is approximately 5e9 vp/mL to 3e8 vp/mL. The values show a little difference between each serotype. Ensure that the same serotype AAV standard is used to measure your samples.

Example data

Figure 1. AAV2 capsid titration curve generated using the GenMedi AAV Titration ELISA kit.

The table shows the standard concentrations of AAV 2 capsids (vp/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 vp/ml	OD450
5.00E+09	1.3562
2.50E+09	0.6971
1.25E+09	0.3748
6.25E+08	0.2427
3.13E+08	0.1399
1.56E+08	0.0862
7.81E+07	0.0630
0	0.0431

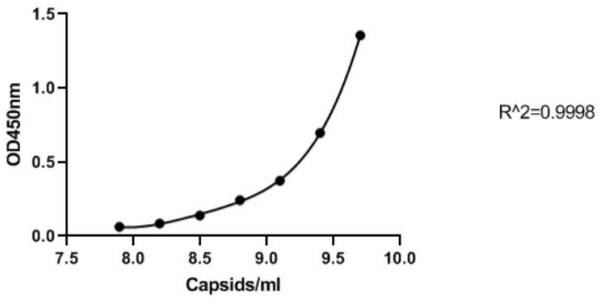


Figure 2. AAV5 capsid titration curve generated using the GenMedi AAV Titration ELISA kit.

The table shows the standard concentrations of AAV 5 capsids (vp/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation ($R^2 = 1.000$) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard vp/ml	OD450
5.00E+09	0.5423
2.50E+09	0.3270
1.25E+09	0.1632
6.25E+08	0.0993
3.13E+08	0.0782
1.56E+08	0.0348
7.81E+07	0.0242
0	0.0143

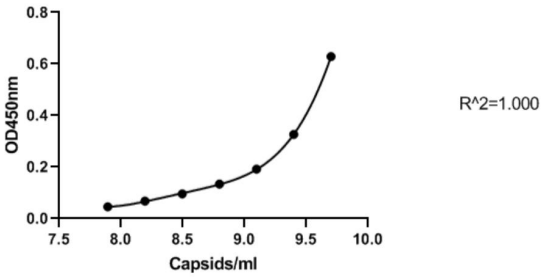


Figure 3. AAV6 capsid titration curve generated using the GenMedi AAV Titration ELISA kit.

The table shows the standard concentrations of AAV 6 capsids (vp/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation ($R^2 = 0.9997$) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard vp/ml	OD450
5.00E+09	1.3626
2.50E+09	0.7555
1.25E+09	0.3669
6.25E+08	0.1925
3.13E+08	0.1039
1.56E+08	0.0604
7.81E+07	0.0441
0	0.0177

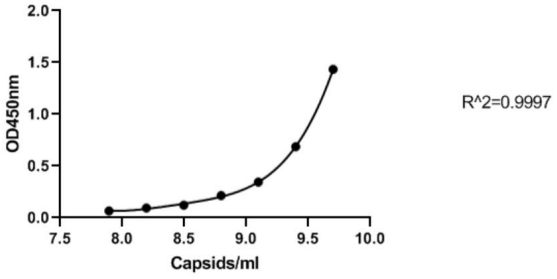


Figure 4. AAV8 capsid titration curve generated using the GenMedi AAV Titration ELISA kit.

The table shows the standard concentrations of AAV 8 capsids (vp/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation ($R^2 = 0.9997$) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard vp/ml	OD450
5.00E+09	1.4574
2.50E+09	0.8019
1.25E+09	0.4440
6.25E+08	0.2127
3.13E+08	0.1064
1.56E+08	0.0609
7.81E+07	0.0366
0	0.0183

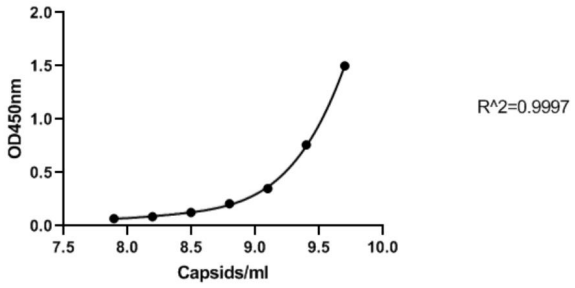


Figure 5. AAV9 capsid titration curve generated using the GenMedi AAV Titration ELISA kit.

The table shows the standard concentrations of AAV 9 capsids (vp/ml) and their corresponding optical density (OD450) readings. The graph plots OD450 values against capsid concentrations, demonstrating a strong correlation ($R^2 = 1.000$) between capsid quantity and absorbance, confirming the assay's sensitivity and accuracy.

Standard vp/ml	OD450
5.00E+09	2.4101
2.50E+09	1.3608
1.25E+09	0.7090
6.25E+08	0.3629
3.13E+08	0.1978
1.56E+08	0.1044
7.81E+07	0.0574
0	0.0140