AAV1 Titration ELISA Kit

Catalog No. EA200068

Principle of the Assay

Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses which have been widely used as viral vectors for research and gene therapy. The virus transduces various dividing and non-dividing cells showing long-term gene expression with low cellular immune response. OriGene's AAV1 Titration ELISA offers a fast, sensitive, and reproducible method for titration of intact AAV1 wild-type virions, AAV1 recombinant virions, as well as assembled and intact empty AAV1 capsids.
This sandwich Enzyme-linked immunosorbent assay (ELISA) is for quantitatively determining AAV serotype 1 particles in cell culture supernatants and purified virus samples. Microtitration wells coated with anti-AAV1 capture antibody are exposed to test samples. The AAV1 particle in the sample is specifically captured onto the immobilized antibody during sample incubation. The captured AAV1 particle is then reacted with a biotinylated AAV1 detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After washing, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the titer of the AAV1 particle present in a sample.

Materials Supplied

Description	Quantity
AAV1 Monoclonal Antibody Coated 96-well Plate in foil pouch with desiccant	1
Lyophilized AAV1 control standard	3 vials
Biotinylated AAV1 Detection Antibody (1x)	14 mL
Streptavidin -HRP Conjugate (1x)	14 mL
Assay Buffer	40 mL
Substrate Solution (TMB)	14 mL
Stop Solution (1N HCl)	14 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

Additional Materials not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

Storage and Stability

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips and the desiccant should be returned to their original foil pouch, which can be kept for 4 weeks at 2-8°C.The AAV1 control standard is stable for 4 weeks at 2 – 8°C after reconstitution. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8˚C. It is recommended that the Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

Indications of Deterioration

The AAV1 Assay kit may be considered to have deteriorated if:

1. Reagents are visibly cloudy.

2. The Substrate Solution turns blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Precautions

1. The reagents supplied in this kit are for ***Research use only***.

2. Keep in mind that the samples you are working with contain infectious virus. Follow the Centers for Disease Control & Prevention and the NIH guidelines to handle potentially infectious agents at the Bio safety Level 2.

3. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to bloodborne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

4. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in case of contact with skin or eyes. Obtain medical attention if necessary.

Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross-contaminate reagents.
4. Some reagents in the AAV1 ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.

6. Warm up the foil bag to room temperature before opening.
7. All reagents should be added to the plate in the same order.
8. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the well changes to yellow (avoid bubbles during this step).
9. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow the manufacturer's recommendations.
10. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

Rinse Cycle

Discard or aspirate content of microtiter strips. Fill each well with Wash Buffer (300µL) using a squirt bottle, manifold dispenser, or automatic plate washer. Incubate approximately 5 seconds and then discard or aspirate content of microtiter strips. Complete removal of liquid at each step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

Preparation for the Assay

1. *Standard preparation*: Reconstitute one vial of lyophilized AAV1 control with 490 µl of distilled water. This will give a final titer of 10x10^8 capsids/mL as shown in Table 1. Make 2x serial dilution of Standard 1 using **Assay Buffer** to generate a standard titer range of 0.16 to 10 x10^8 capsids/mL.

2. *Sample preparation*: AAV1 titer must be estimated prior to performing the full experiment by testing a serially diluted representative sample using **Assay Buffer**. Select an optimal dilution level such that the final titer falls near the middle of the assay linear dynamic range.

3. *Wash buffer*: Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficiently for immediate needs.

Table 1: AAV1 Control Standard Curve Generation

Standard Number	Titer of AAV1 (x10^8 capsids/mL)	AAV1 Control (µL)	Assay Buffer (µL)
1	10	490	0
2	5	240 of #1	240
3	2.5	240 of #2	240
4	1.25	240 of #3	240
5	0.625	240 of #4	240
6	0.313	240 of #5	240
7	0.156	240 of #6	240
8	0		240

Assay Procedure

Note: All standards and samples should be tested in duplicate.

1. Allow all reagents to reach room temperature (18-25˚C).

2. Select sufficient microtitration well strips to accommodate all test samples, control standards and reagent blank. Fit the strips into the holding frame.

3. Dispense 100 µL of each standard and sample into appropriate wells.

4. Incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

6. Add 100 µL of Biotinylated AAV1 Detection Antibody into each well and incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

8. Add 100 µL of Streptavidin HRP conjugate into each well and incubate for 25 minutes at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

9. Wash the microtitration plate 5 times as described in the Rinse Cycle section.

10. Dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25˚C) and protected from direct sunlight for 15 minutes.

11. Stop the reaction by adding 100 µL of Stop Solution to each well. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

12. Read the absorbance values at 450 nm using a microplate reader. If wavelength correction is available, set it to 540 nm or 570 nm.

Calculation of Results

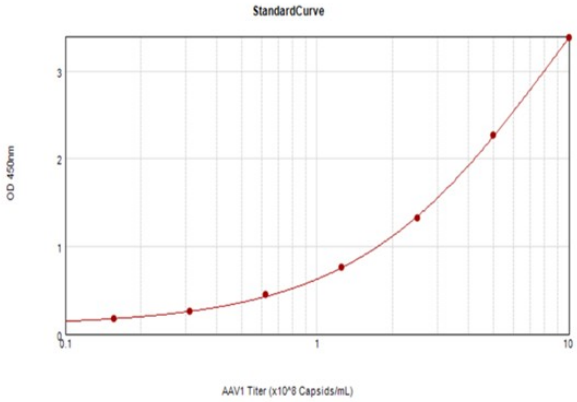
Average the duplicate readings for each standard and sample. A 4-parameter logistic (4-PL) regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) regression curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

Table 2. Example Data at 450nm.

Standards	OD at 450 nm
Standard 1 (10x10^8 capsids/mL)	3.3791
Standard 2 (5x10^8 capsids/mL)	2.2714
Standard 3 (2.55x10^8 capsids/mL)	1.3226
Standard 4 (1.25x10^8 capsids/mL)	0.7575
Standard 5 (0.63x10^8 capsids/mL)	0.45
Standard 6 (0.31x10^8 capsids/mL)	0.2619
Standard 7 (0.16x10^8 capsids/mL)	0.1709
Standard 8 (0 capsids/mL)	0.0957

Typical AAV1 Titration ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only.



Note: This standard curve is only an example and should not be used to generate any results.

Performance Characteristics

1. **Recovery**
   The recovery of AAV1 spiked to three different levels of the assay range in diluted samples was evaluated.

Sample	Average Recovery	Range
Culture Media	102%	99-108%

2. **Linearity**
   To assess the linearity of the assay, AAV1 spiked samples were diluted to produce samples with values within the dynamic range of the assay.

	Cell culture media
%Expected at 1:2 dilution	94
%Expected at 1:4 dilution	94
%Expected at 1:8 dilution	92

3. **Sensitivity (LLOD):** 9x10^6 capsids/mL

4. **Specificity:** Minimal cross-reactivity with AAV6 was found. The AAV1 Titration ELISA was performed according to the manual. Empty AAV capsid preparations of serotypes 1 to 9 and AAVrh10 with respective concentrations were applied to the microtiter plate.

OD values for all other AAV serotypes were within the background range except the OD values for AAV1 and AAV6 capsids were above the background OD (Table 2).

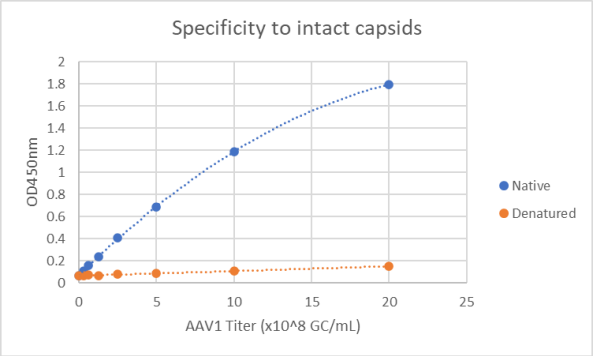
Table 2. OD values for all AAV serotypes

Serotype	Titer [GC/ml]	OD450nm
AAV1	2.50E+09	2.063
AAV2	2.00E+09	0
AAV3	2.00E+09	0
AAV5	2.00E+09	0
AAV6	2.00E+09	0.1608
AAV7	2.00E+09	0.0058
AAV8	2.00E+09	0
AAV9	2.00E+09	0
AAVrh10	2.00E+09	0.0028
Background	0.00E+00	0

Note: AAV Titer (GC/ml) is determined by qPCR.

5. **Specific recognition of fully assembled capsids**

To demonstrate the selective detection of fully assembled AAV1 capsids, recognition of native and denatured AAV1 capsids was analyzed by AAV1 Titration ELISA. The denatured AAV1 capsids were pre-treated by boiling 5 min at 95°C. AAV1 detection antibody exhibits binding to native AAV1 capsids, but not to denatured AAV1 capsids confirming the selective detection of native AAV1 capsids.



6 **Precision**

Culture media samples with different levels of AAV1 were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Intra-assay:

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
AAV1 sample1 (n=10)	2.52	2.19	2.33	2.35
AAV1 sample2 (n=10)	3.22	4.23	4.30	3.92
AAV1 sample3 (n=10)	8.20	7.07	7.60	7.62

Inter-assay

Samples	AAV1 sample1 (n=10)	AAV1 sample2 (n=10)	AAV1 sample3 (n=10)
Mean (capsids/ml) in assay1	1.86x10^8	0.91x10^8	0.50x10^8
Mean (capsids/ml) in assay2	1.87x10^8	0.89x10^8	0.48x10^8
Mean (capsids/ml) in assay3	1.82x10^8	0.88x10^8	0.49x10^8
Ave (capsids/ml)	1.85x10^8	0.89x10^8	0.49x10^8
SD	0.02	0.01	0.01
%CV	1.33	1.22	2.45

Limitations of Use

1. This kit is for research use only, **not for use in diagnostic procedures.**
2. The AAV1 titer measured using OriGene AAV1 Titration ELISA kit may not be interchangeable with that obtained from other assay kits.
3. The assay cannot be used to quantify samples with values higher than the highest standard without further dilution of the samples.

Assay Flowchart