Version：01
Update (MM/DD/YYYY)：03/28/2025

Product Manual

AAV8 Titer Capsid ELISA Kit

Cat. No. L01051

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read the technical manual carefully before using this product.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

Contents

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

I. DESCRIPTION

GenScript AAV8 Titer Capsid ELISA Kit (Cat. No. L01051) is a Sandwich ELISA Kit that is designed for quantitatively measuring the total capsid titer of adeno-associated virus serotype 8 (AAV8) in testing samples during viral vector production. This ELISA kit provides an easy, accurate, and reproducible method for AAV8 titration.

Adeno-associated viruses (AAV) are now widely-used viral vectors for gene therapy, with AAV8 being one of the widely studied serotypes. When using AAV8 as the viral vector in gene therapy, determination of the total capsid titer of the purified AAV8 particle product is an essential characterization procedure, and represents a critical step in clinical applications.

II. ASSAY PRINCIPLE

AAV8 Titer Capsid ELISA Kit is based on the sandwich ELISA method. When standards or samples are added to the capture plate, the AAV8 capsids in the sample can be captured on the plate coated with the capture antibody. Then the detection antibody (Biotin Anti-AAV8 Antibody) conjugated with biotin is added to interact with the AAV8 capsids bound on the plate. Streptavidin-Horseradish Peroxidase conjugate (Streptavidin-HRP) is added to interact with the biotin conjugated detection antibody. After washing steps, 3,3',5,5'-Tetramethylbenzidine solution (TMB) is added resulting in formation of blue color. The reaction is stopped by adding Stop Solution. Application of the Stop Solution results in the color changing from blue to yellow. The intensity of the color can be read at 450 nm by a microplate reader. The quantity of AAV8 capsids in the sample is precisely quantified against an AAV8 standard curve.

III. KIT CONTENTS

The kit provides the following reagents.

Component Name	Quantity/Size	Part No.
AAV8 Standard	3 vials (1.41E+09 capsids/mL)	AV8-MM00B-1
20× Detection Antibody	1 × 750µL	AV8-MM00B-2
Pre-coated plate	1 × 96-well plate	AV8-MM00B-3
HRP Conjugate	1 × 15mL	AV8-MM00B-4
20× Assay Buffer	2 × 30mL	AV8-MM00B-5
TMB	1 × 15mL	AV8-MM00B-6
Stop Solution	1 × 10mL	AV8-MM00B-7

- Capture Plate: 96 well microplates (8 wells x 12 strips); 12 strips are configured in plate; plate is sealed in a foil pouch with a desiccant.
- The kit does not include a paper manual. Please download the latest version of the manual from GenScript's official website.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

IV. STORAGE

Store kit components at 2-8°C. The unopened kit is valid for 12 months from the production date. Reconstituted AAV8 standard stable at 2-8°C for 2 weeks. -20°C or below for long-term storage and avoid repeated freeze-thaw cycles.

V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Microplate reader capable of measuring absorbance at 450 nm
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder
- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes
- 10 µL, 200 µL, and 1000 µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator
- Centrifuge
- 37 ± 2 °C Incubator

VI. PRECAUTIONS

1. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials in accordance with local regulations. For more detailed information, please get in touch with your local lab safety committee.
2. The microplate has detachable strips, so do not touch the bottom of the well while disassembling them.
3. Do not leave the plate too long after each wash to avoid the plate drying out.
4. 20× Assay Buffer may precipitate at 4°C due to high salt concentration, and the precipitates can be redissolved at room temperature.
5. Do not use this kit with components from other commercial kits, and do not mix components from different batches of kits. A standard curve must be prepared for each plate, and duplicates are recommended.
6. All reagents must be equilibrated to room temperature (18-25°C) before use. The TMB

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

substrate should be warmed to 37°C before use.

7. Make sure there is no liquid left in each well after each wash.
8. Using a plate washer can reduce the experimental error. For manual washing it is recommended to soak the plate in 1× Assay Buffer for 1 minute after each addition.
9. The TMB substrate incubation needs to be protected from light and strictly controlled within 10 minutes.
10. The Stop Solution contains sulfuric acid and may cause skin or eye burn. Rinse immediately with plenty of water and seek medical assistance if necessary.
11. When dissolving the standard,mix it gently and avoid vortexing.
12. This product is for scientific research only and may not be used for other purposes.

VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. Sample stability has not been evaluated. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria that meet their needs.
2. Store specimens at -20°C or lower if not testing immediately. Avoid repeated freeze-thaw cycles.

VIII. PROTOCOL

- **Reagent Preparation**

  All reagents must be equilibrated to room temperature before use (20°C to 25°C). All samples and reagents, should be vortexed before use. Store all reagents back in refrigerator promptly after use.

  1× Assay Buffer: Dilute the 20× Assay Buffer with deionized or distilled water in a volume ratio of 1:19. Store the solution at 2°C to 8°C when not in use. Store the solution at 2°C to 8°C when not in use.

  1× Detection Antibody: Dilute the 20× Detection Antibody with 1× Assay Buffer with a volume ratio of 1:19. Store the solution at 2°C to 8°C when not in use.

  Note: If any precipitate is found in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

  Sample preparation

  Unknown samples, especially samples with very high titer of AAV8, must be diluted prior to the assay to obtain an accurate titer value of AAV8 which is within the linear range of the kit. We recommend several serial 10-fold dilutions with 1× Assay Buffer for unknown samples to ensure that at least one diluted sample is in the range of the standard curve.

  It is recommended that all samples be prepared in duplicate. The results are multiplied by the

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

dilution factor to determine the AAV8 value in the original sample.

Standard working solution

Preparation of AAV8 capsid standards: Add 700 µL of distilled H_2O to one vial of standard. Dissolve at room temperature for 10-20 min, mix gently and avoid vortexing. The titer of the reconstituted standard is 1.41E+09 capsids/mL. Two-fold serial dilution of the AAV8 standard with 1× Assay Buffer for the titration curve in duplicates is recommended.

Standard	Concentration (capsids/mL)	Final Dilution
Std-1	1.41E+09	Undiluted
Std-2	7.04E+08	1:2
Std-3	3.52E+08	1:4
Std-4	1.76E+08	1:8
Std-5	8.80E+07	1:16
Std-6	4.40E+07	1:32
Std-7	2.20E+07	1:64
1× Assay Buffer	0	N/A

- **Capture Plate Preparation**

It is recommended that all standards be prepared at least in duplicate.

Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

Table 1. Setup of AAV8 standards and samples on Capture Plate

	Standards		Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std-1	Std-1	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	Std-2	Std-2	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	Std-3	Std-3	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	Std-4	Std-4	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	Std-5	Std-5	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	Std-6	Std-6	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	Std-7	Std-7	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

H	1× Assay Buffer	1× Assay Buffer	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Std： Standard number；**S：** Sample number

- **Test Procedure**

Standards and Samples Incubation

1. Take out the 96-well plate, seal unused strips and immediately put back at 2-8°C.
2. Wash the plate with 1× Assay buffer. Pat dry.
3. Add 100 µL of standard working solution and samples to the corresponding wells in the Capture Plate.
4. Cover the plate with Plate Sealer and incubate at 37°C for 60 minutes.
5. Remove the Plate Sealer and wash the 96-well plate with 300 µL 1× Assay buffer 3 times.
6. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps and pat dry.

Detection Antibody Incubation

7. Add 100 µL of 1× detection antibody to all the testing wells.
8. Cover the plate with Plate Sealer and incubate at 37°C for 60 minutes.
9. Remove the Plate Sealer and wash the 96-well plate with 300 µL 1× Assay buffer 3 times.
10. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps and pat dry.

Enzyme Conjugate Incubation

11. Add 100 µL of Streptavidin-HRP to all the testing wells.
12. Cover the Plate with Plate Sealer and incubate at 37°C for 60 min.
13. Remove the Plate Sealer and wash the 96-well plate with 300 µL 1× Assay buffer 3 times.
14. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps and pat dry.

Substrate Reaction and Absorbance Measurement

15. Add 100 µL of TMB Substrate to each well. Incubate at 37°C in the darkness for 10 min. It is recommended to preheat the TMB Substrate to 37°C.
16. Add 50 µL of Stop Solution to each well and mix gently.
17. Immediately read the OD value of each well at 450nm by a microplate reader. It is recommended to read the OD450 values within 5 min after adding the Stop Solution.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

IX. INTERPRETATION OF RESULTS

- **Suggested Calculation of Data**

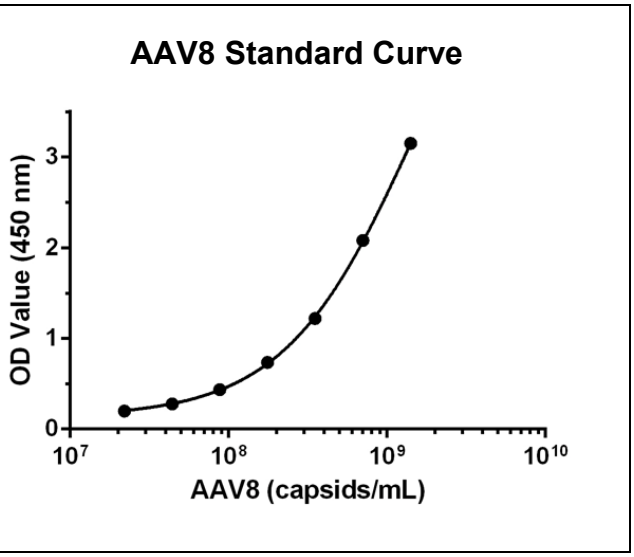
  Statistical software can be used to create the standard curves. Choose a method with high Goodness of Fit (R^2) to analyze the data, such as a 4- or 5-parameter logistic model that provides point-to-point curve fitting.

  Calculate the sample titer by entering the sample OD450 value into the equation for the standard curve. If you diluted the sample, multiply by the dilution factor. The lower limit of quantitation (LOQ) is 2.80E+07 capsids/mL. If the OD450 of the sample falls above the valid linear range, the sample should be further diluted and retested.

  The standard curve has been provided for demonstration only. It should be prepared each time an assay is performed.

Table 2. A typical sample data for the AAV8 standard curve.

AAV8 titer (capsids/mL)	OD450
1.41E+09	3.15
7.04E+08	2.08
3.52E+08	1.22
1.76E+08	0.737
8.80E+07	0.437
4.40E+07	0.278
2.20E+07	0.201
0	0.106



A typical example of AAV8 standard curve using a four-parameter logistic curve. Each standard was analyzed in duplicate. The R-Square of this curve is 0.99.

X. ANALYTICAL PERFORMANCE

- **Detection Range**

  The detection range of this kit is from 2.20E+07 to 1.41E+09 capsids/mL.

- **Sensitivity**

  The sensitivity of this kit is 1.10E+07 capsids/mL.

- **Measurement Precision**

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

CV < 10%

XI. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate is contaminated	Use new Substrate from the same Lot
	Volumes of reagents are not correct	Repeat assay with the required volumes as noted in the manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat the assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	Substrate is contaminated	Use new substrate from the same Lot
	Evaporation of wells during incubations	Perform incubation steps with a plate sealer in a repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay

For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.