Version 02
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# Product Manual

# AAV2 Titer Capsid ELISA Kit

## Cat. No. L00942

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

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

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# Contents





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## I. DESCRIPTION

GenScript AAV2 Titer Capsid ELISA Kit (Cat. No. L00942) is a Sandwich ELISA Kit that is designed for quantitatively measuring the total capsid titer of adeno-associated virus serotype 2 (AAV2) in testing samples during viral vector production. The total capsid titer stands for the amount of all fully assembled viral capsids, including full and empty AAV2 capsids. This ELISA kit provides an easy, accurate, and reproducible method for AAV2 titration.

Adeno-associated viruses (AAV) are now widely-used viral vectors for gene therapy, with AAV2 being the most extensively studied serotype. When using AAV2 as the viral vector in gene therapy, determination of the total capsid titer of the purified AAV2 particle product is an essential characterization procedure, and represents a critical step in clinical applications.

## II. ASSAY PRINCIPLE

AAV2 Titer Capsid ELISA Kit is based on the sandwich ELISA method. When standards (AAV2 Control) or samples are added to the capture plate, the AAV2 capsids in the sample can be captured on the plate coated with the capture antibody. Then the detection antibody (Biotin Anti-AAV2 Antibody) conjugated with biotin is added to interact with the AAV2 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 Solution) 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 and 650 nm by a microplate reader. The quantity of AAV2 capsids in the sample is precisely quantified against an AAV2 standard curve.

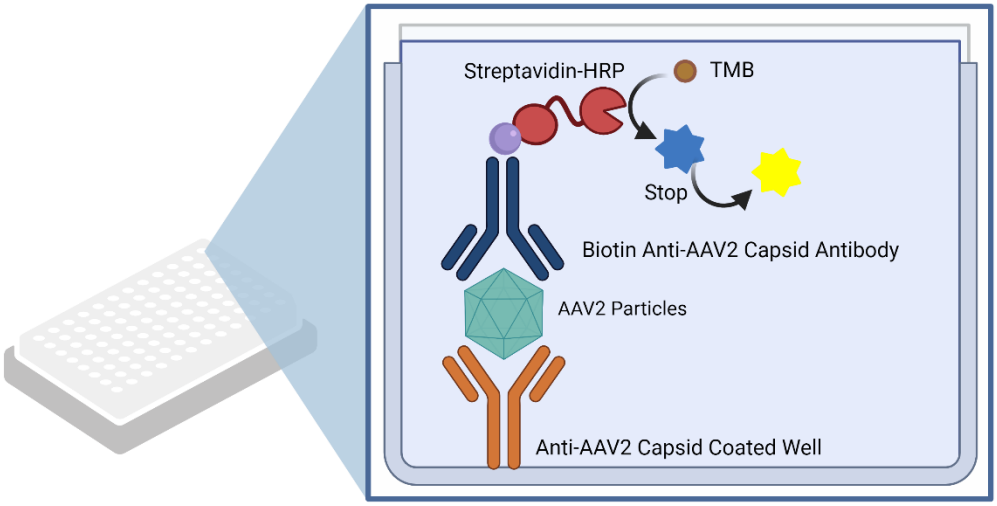


**Figure 1:** Schematic diagram of AAV2 Titer Capsid ELISA Kit



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## III. KIT CONTENTS

The kit provides the following reagents and items.

| Component | Quantity/Size | Part No. |
|---|---|---|
| Capture Plate | 1 plate | G1-80 |
| AAV2 Control | 2 vials (Specified on product label) | G1-10 |
| Biotin Anti-AAV2 Antibody | 1 bottle (12 mL) | G1-20 |
| Streptavidin-HRP | 1 bottle (12 mL) | E1-30 |
| Sample Dilution Buffer | 1 bottle (50 mL) | G1-60 |
| TMB Solution | 1 bottle (12 mL) | A1-40 |
| 20× Wash Solution | 1 bottle (40 mL) | A1-70 |
| Stop Solution | 1 bottle (6 mL) | A1-50 |
| Plate Sealer | 2 pieces | N/A |

- 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.
- AAV2 Control is two vials of lyophilized powder containing empty AAV2 capsids. It needs to be reconstituted with deionized or distilled water before use. The number of empty AAV2 capsids in each batch of AAV2 Control is different, and this information is specified on the product label. The reconstituted AAV2 Control can be stored at 2°C to 8°C with the kit for up to 2 weeks. For long term storage, please aliquot and store at -20°C or below. Avoid repeated freezing and thawing cycles.

## IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

## 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



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- 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 contact your local lab safety committee.
2. All personnel working with AAV2 must complete appropriate Biosafety Training.
3. Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
4. Avoid contact of skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep container tightly closed. In case of an accident, please seek medical assistance immediately.
5. Do not use the kit if there is any visible damage to the packaging or kit contents.
6. Do not mix components from different batches. Do not mix with components from other manufacturers.
7. Do not use reagents beyond the stated expiry date.
8. All reagents must be equilibrated to room temperature (20° - 25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contamination may occur.
9. Before opening the AAV2 Control, quickly spin the vial to ensure that all the lyophilized powder has collected at the bottom, and prevent the lyophilized powder from splashing when opening the lid.
10. Use only distilled or deionized water and clean glassware.
11. Do not let wells dry during the test; add reagents immediately after completing washing steps.
12. The kit has not been validated with AAV3 capsids and may cross-react with AAV3 capsid. It should not be used without prior standardization.



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## 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, except AAV2 Control, should be vortexed before use. Store all reagents back in refrigerator promptly after use.

  **1× Wash Solution:** Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. 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 AAV2, must be diluted prior to the assay to obtain an accurate titer value of AAV2 which is within the linear range of the kit. We recommend several serial 10-fold dilutions for unknown samples to ensure that at least one diluted sample is in the range of the standard curve. Usually 10-10,000 fold dilutions of the sample are required.

  It is recommended that all samples be prepared in duplicate. The results are multiplied by the dilution factor to determine the AAV2 value in the original sample.

  **Standard working solution**

  Reconstitute AAV2 Control with 500 µL of deionized or distilled water to make standard working solution. Mix standard working solution by rolling and incubate for 5 min at RT. **Avoid vortex**. Find the amount of capsids/mL on the label.

  Dilute standard working solution with Sample Dilution Buffer with a volume ratio of 1:10. For example, mix 100 µL of standard working solution with 900 µL of Sample Dilution Buffer to make 1 mL of standard working solution with label of “Std-01”. We recommend to dilute the standard working solution with Sample Dilution Buffer in steps of 1:2.

  Prepare seven 1.5 mL tubes labeled numerically from Std-02 to Std-08 consecutively, and



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pipette 500 µL of Sample Dilution Buffer into each tube.

Pipette 500 µL of standard solution from Std-01 tube into the Std-02 tube and mix. Then pipette 500 µL of the solution from the former tube into the latter one according to these steps.

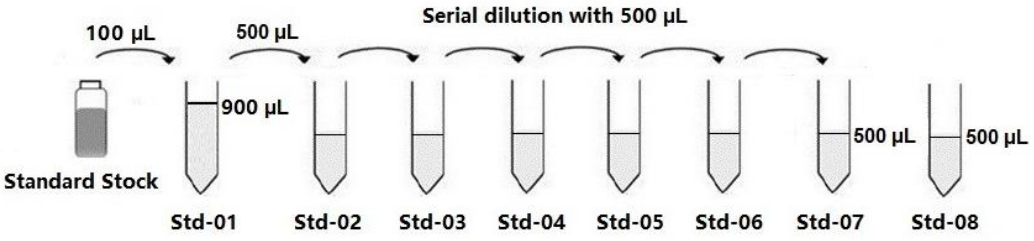


**Figure 2:** Diagram of Serial Dilution.

- **Capture Plate Preparation**

1. It is recommended that all standards be prepared at least in duplicate.
2. 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.
3. 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 AAV2 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 |
| H | Std-8 | Std-8 | S8 | S16 | S24 | S32 | S40 | S48 | S56 | S64 | S72 | S80 |

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

- **Test Procedure**

**Standards and Samples Incubation**

1. Add 100 µL of standard working solution and samples to the corresponding wells in the Capture Plate.



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2. Cover the plate with Plate Sealer and incubate at 37°C for 60 minutes.
3. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution four times.
4. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after the wash steps.

**Detection Antibody Incubation**

5. Add 100 µL of Biotin Anti-AAV2 Antibody to all the testing wells.
6. Cover the plate with Plate Sealer and incubate at 37°C for 60 minutes.
7. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution four times.
8. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps.

**Enzyme Conjugate Incubation**

9. Add 100 µL of Streptavidin-HRP to all the testing wells.
10. Cover the Plate with Plate Sealer and incubate at 37°C for 10 minutes.
11. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution four times.
12. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after wash steps.

**Substrate Reaction and Absorbance Measurement**

13. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
14. Add 50 µL of Stop Solution to each well to stop the reaction.
15. Read the absorbance in microplate reader at dual wavelengths of 450 nm and 650 nm immediately. Then the absorbance at 650 nm which serves as the reference is subtracted from the absorbance of the same well measured at 450 nm to eliminate the background signal that comes from turbidity.



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## IX. ASSAY PROCEDURE SUMMARY

1. • Dilute the test samples and a set of standards with Sample Dilution Buffer.
2. • Add 100 µL of the diluted samples/standards to the corresponding wells. Incubate the plate at 37°C for 60 minutes.
3. • Wash the plate with 260 µL of 1× Wash Solution per well four times.
4. • Add 100 µL of the Biotin Anti-AAV2 Antibody to the well and incubate at 37°C for 60 minutes.
5. • Wash the plate with 260 µL of 1× Wash Solution per well four times.
6. • Add 100 µL of the Streptavidin-HRP and incubate at 37°C for 10 minutes.
7. • Wash the plate with 260 µL of 1× Wash Solution per well four times.
8. • Add 100 µL of TMB Solution and incubate the plate in dark at 25°C for 15 minutes.
9. • Add 50 µL of Stop Solution to each well to stop the reaction.
10. • Read the plate immediately.



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## X. INTERPRETATION OF RESULTS

- **Assay Validation**

To ensure the validity of results, the following criteria noted in the Table 2 are required. If a test fails to meet the requirements, the test is invalid and must be repeated.

**Table 2**. OD 450/650 nm values for quality control

| Items | Special Test | Requirements |
|---|---|---|
| 1 | OD 450/650 nm value for Std-01 | > 2.0 |
| 2 | OD 450/650 nm value for Std-08 | < 0.1 |

*Note： The standards in the table are only intended to evaluate the performance of the kit.*

- **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 linear regression or a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

**Manual method for demonstration:**

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

1. Plot standard curve with the AAV2 concentration (capsids/mL) on the x-axis and the corresponding mean absorbance value on the y-axis.
2. Using a four-parameter logistic curve fitting program, calculate the best fitting linear line through the points of the standard curve.

The equation for the model is:

$$y = D + \frac{A - D}{1 + (\frac{x}{C})^B}$$

A = the minimum value that can be obtained (i.e. what happens at 0 dose)

D = the maximum value that can be obtained (i.e. what happens at infinite dose)

C = the point of inflection (i.e. the point on the S shaped curve halfway between a and d)

B = Hill's slope of the curve (i.e. this is related to the steepness of the curve at point c)

3. Determine the p24 concentration (pg/mL) for the samples with the standard curve.



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**Table 3**. Sample data for standard curve.

| Standard | Absorbance (450/650 nm) | | |
|---|---|---|---|
| (Capsids/mL) | Duplicate 1 | Duplicate 2 | Average |
| 2.57E+09 | 3.0787 | 3.2939 | 3.1863 |
| 1.28E+09 | 1.7633 | 1.8283 | 1.7958 |
| 6.42E+08 | 0.876 | 0.9312 | 0.9036 |
| 3.21E+08 | 0.4377 | 0.4749 | 0.4563 |
| 1.60E+08 | 0.2379 | 0.2572 | 0.24755 |
| 8.02E+07 | 0.13 | 0.1442 | 0.1371 |
| 4.01E+07 | 0.0814 | 0.0852 | 0.0833 |
| 0.00E+00 | 0.0307 | 0.0306 | 0.03065 |

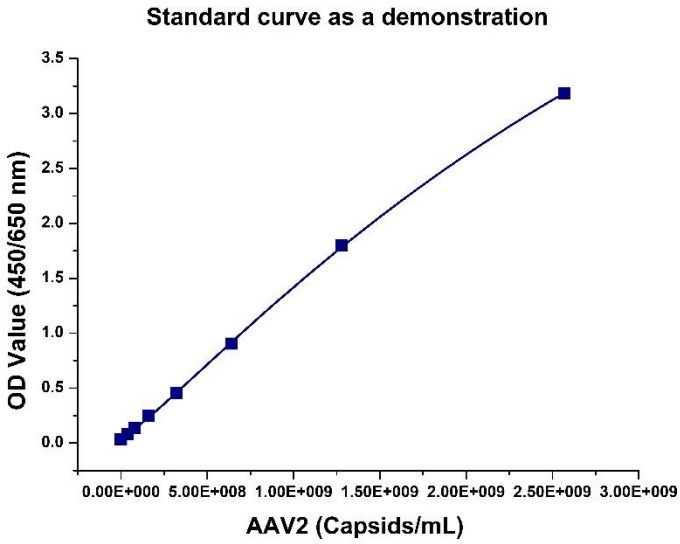


**Figure 3:** AAV2 Titer Capsid ELISA kit standard curve.

## XI. ANALYTICAL PERFORMANCE

- **Linear Range**

  The linear range of this kit is from 4.8E+07 to 2.5E+09 capsids/mL according to the evaluation method recommended in CLSI guideline EP6-A [2].

- **Detection Capability**

  According to the evaluation method recommended in CLSI guideline EP17-A2[3], the LoB (Limit of Blank) of this kit is 1.31E+07 capsids/mL, the LoD (Limit of Detection) is 2.08E+07 capsids/mL and the LoQ (Limit of Quantitation) is 2.15E+07 capsids/mL.



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- **Measurement Precision**

Intra- and inter-assay precision were measured in 3 pools of different concentrations, using 3 lots of kits.

**Table 4:** Intra-assay CV evaluation with AAV2 Titer Capsid ELISA Kit

| Repeats | Average Conc. (capsids/mL) | % CV |
|---|---|---|
| 10 | 1.65E+08 | 3% |
| 10 | 5.15E+08 | 2% |
| 10 | 1.99E+09 | 3% |

**Table 5:** Inter-assay CV evaluation with three-batch AAV2 Titer Capsid ELISA Kit

| Batch Amount | Repeats | Average Conc. (capsids/mL) | % CV |
|---|---|---|---|
| 3 | 3×10 | 1.47E+08 | 9% |
| 3 | 3×10 | 4.83E+08 | 5% |
| 3 | 3×10 | 1.88E+09 | 6% |

- **Specificity/Cross-Reactivity**

The AAV2 Titer Capsid ELISA Kit was performed according to this manual. As samples, empty AAV capsid preparations of serotypes 1, 2, 5, 6, 8, 9, and DJ with respective concentrations were applied to the microplate. The following ODs were obtained:

**Table 6:** AAV serotype cross-reactivity of the AAV2 Titer Capsid ELISA Kit

| Serotype | Capsids Titer | Absorbance (450/650 nm) | | |
|---|---|---|---|---|
| | (Capsids/mL) | Duplicate 1 | Duplicate 2 | Average |
| AAV 1 | 1.07E+10 | 0.0124 | 0.0118 | 0.0121 |
| AAV 2 | 1.20E+10 | 4.975 | 5.0119 | 4.99345 |
| AAV 5 | 4.29E+10 | 0.0149 | 0.0128 | 0.01385 |
| AAV 6 | 3.13E+10 | 0.0169 | 0.0153 | 0.0161 |
| AAV 8 | 2.31E+10 | 0.0162 | 0.013 | 0.0146 |
| AAV 9 | 1.22E+10 | 0.0138 | 0.014 | 0.0139 |
| AAV DJ | 1.48E+10 | 0.0138 | 0.0131 | 0.01345 |
| Negative control | 0 | 0.0134 | 0.0114 | 0.0124 |



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## XII. 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 |



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## XIII. REFERENCES


1. CLSI document EP06-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2nd Edition).
2. CLSI document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition).
3. CLSI document EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition).







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