

AAV5 Titration ELISA Kit

Pack Size: 96 tests

Catalog Number: AAV-A005

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

AAV5 Titration ELISA Kit was developed for the detection and quantitative determination of AAV5 capsid titration in AAV gene therapy product preparation processing. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of AAV5 capsid by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-AAV5 Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-AAV5 Antibody to the plate, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of AAV5 capsid present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of AAV5 capsid bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
AAV05-C01	Pre-coated Anti-AAV5 Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
AAV05-C02	AAV5 Standard	8.7E+10 capsids	Powder	2-8°C	-70°C
AAV05-C03	Biotin-Anti-AAV5 Antibody	20 µg	Powder	2-8°C	-70°C
AAV05-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
AAV05-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
AAV05-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
AAV05-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

AAV05-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C
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REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Centrifuge;

37°C Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10× Washing Buffer;

SHIPPING AND STORAGE

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37 °C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. According to the information provided in Table 2, the lyophilized was reconstructed with PBS as place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70°C. AAV05-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 40µL. AAV05-C03 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
AAV05-C02	AAV5 Standard	8.70E+10 capsids	8.70E+11 capsids/mL	100 µL 1xPBS
AAV05-C03	Biotin-Anti-AAV5 Antibody	20 µg	200 µg/mL	100 µL 1xPBS

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti- AAV5 Antibody working fluid:

Dilute Biotin-Anti- AAV5 Antibody to 0.5 µg/mL with Dilution Buffer. Please prepare it for one-time use only.

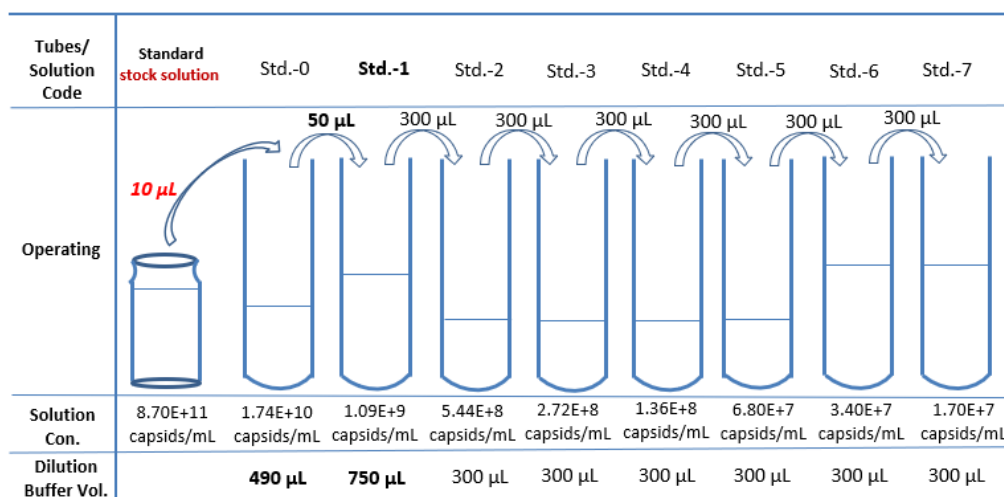
1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

Make serial dilutions of the AAV5 capsid as a Standard curve with Dilution Buffer as recommended in Figure 1.

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE AAV5



3. Add Samples

Add 100µL serially diluted AAV5 Standard curve and samples to each well. For blank Control wells, please add 100µL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

Note: It is recommended to set multiple holes for samples and standard curves to be measured.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels.

Repeat the wash step above for three times.

5. Add Biotin-Anti-AAV5 Antibody

For all wells, add 100 µL **Biotin-Anti-AAV5 Antibody (dilute to 0.5 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 µL **Streptavidin-HRP (1:2000 dilute)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour, avoid light.

8. Washing

Repeat step 4.

9. Substrate Reaction

Add 100 µL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

10. Termination

Add 50 µL **Stop Solution** to each well, and tap the plate gently for 5 min to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

11. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the value read at $OD_{450\text{ nm}}$ with the value read at $OD_{630\text{ nm}}$.

CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: $1.70E+7$ - $1.09E+9$ capsids/mL.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted from the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20°C - 25°C) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C .

TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only.

AAV5 Standard (capsids/mL)	OD450-630nm
1.09E+09	2.179
5.44E+08	1.366
2.72E+08	0.753
1.36E+08	0.421
6.80E+07	0.227
3.40E+07	0.137
1.70E+07	0.097
0	0.050

