A005H-EN.01

# AAV5 Titration ELISA Kit (Fast)

**Pack Size: 96 tests**

**Catalog Number：** AAV-A005H

**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 (Fast) 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 HRP- Anti-AAV5 Antibody 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 |
| AAV05H-C01 | Pre-coated Anti-AAV5 Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| AAV05H-C02 | AAV5 Standard | 8.7E+10 capsids | Powder | 2-8°C | -70°C |
| AAV05H-C03 | HRP-Anti-AAV5 Antibody | 20 μg | Powder | 2-8°C | -70°C |
| AAV05H-C04 | 10xWashing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| AAV05H-C05 | 2xDilution Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| AAV05H-C06 | Substrate Solution | 12 mL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| AAV05H-C07 | Stop Solution | 7 mL | Liquid | 2-8°C | 2-8°C |

## REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED



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

Centrifuge;

37ºC 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 ºC 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. AAV05H-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 20µL. AAV05H-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. |
|---|---|---|---|---|
| AAV05H-C02 | AAV5 Standard | 8.7E+10 capsids | 1.74E+12 capsids/mL | 50 µL 1xPBS |



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| | | | | |
|---|---|---|---|---|
| AAV05H-C03 | HRP-Anti-AAV5 Antibody | 20 μg | 200 ug/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 HRP-Anti-AAV5 Antibody working fluid:

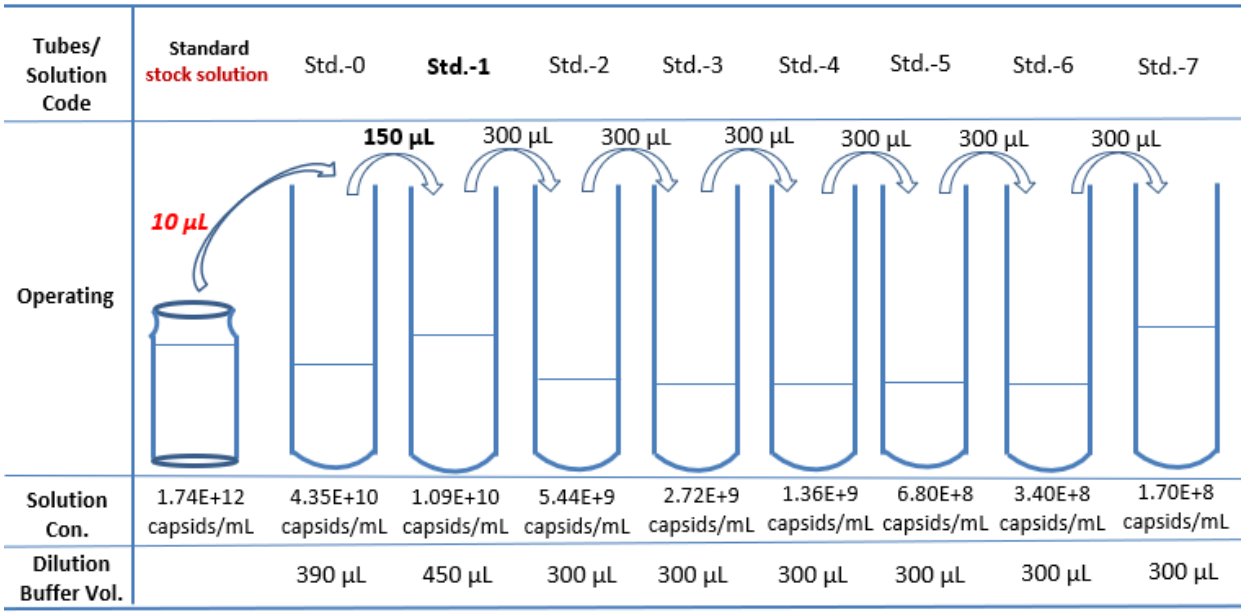
Dilute HRP-Anti-AAV5 Antibody to 0.5 μg/mL with Dilution Buffer. The prepared working fluid should avoid light.

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



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100μL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37℃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 HRP-Anti-AAV5 Antibody

For all wells, add 100 μL **HRP-Anti-AAV5 Antibody (dilute to 0.5 μg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

### 6. 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 37℃ 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* $OD_{450\ nm}$ *with the value read at* $OD_{630\ nm}$.

## CALCULATION OF RESULTS

1. Normal range of Standard curve: R2≥0.9900, detection range: 1.70E+8-1.09E+10 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



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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.

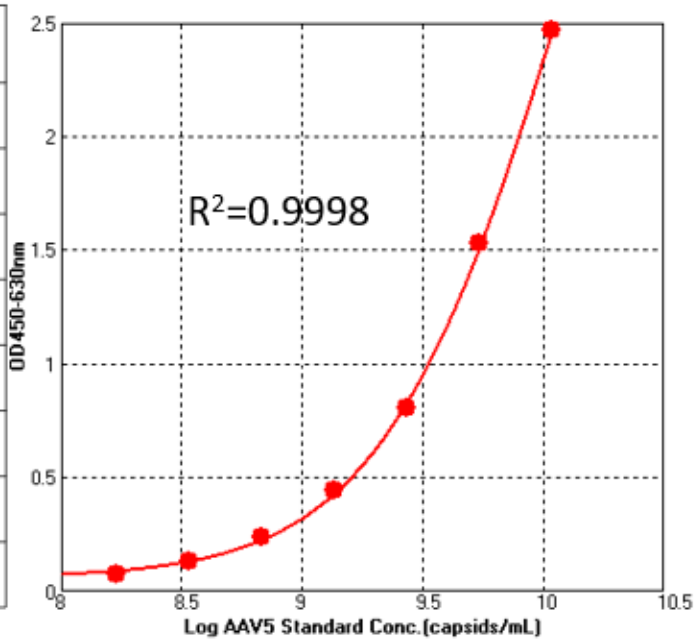
## PRECAUTIONS

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℃-25℃) 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+10 | 2.469 |
| 5.44E+09 | 1.527 |
| 2.72E+09 | 0.807 |
| 1.36E+09 | 0.444 |
| 6.80E+08 | 0.237 |
| 3.40E+08 | 0.132 |
| 1.70E+08 | 0.078 |
| 0 | 0.026 |



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