A008-EN.01

Anti-AAV8 Antibody ELISA Kit

Pack Size: 96 tests

Catalog Number： PAV-A008

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

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

INTENDED USE

Anti-AAV8 Antibody ELISA Kit is developed for the detection of anti-AAV8 antibodies in serum. It can be used for immunogenicity studies and enrollment screening. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of Anti-AAV8 Antibody by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with AAV8 Capsid Protein. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-AAV8 Capsid Protein 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 Anti-AAV8 Antibody 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 Anti-AAV8 Antibody bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
PAV008-C01	Pre-coated AAV8 Capsid Protein Microplate	1 plate	Solid	2-8°C	2-8°C
PAV008-C02	Anti-AAV8 Antibody Standard	2.5 μg	Powder	2-8°C, avoid light	-70°C, avoid light
PAV008-C03	Biotin-AAV8 Capsid Protein	1 μg	Powder	2-8°C, avoid light	-70°C, avoid light
PAV008-C04	Streptavidin-HRP	10 μg	Powder	2-8°C, avoid light	-70°C, avoid light
PAV008-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
PAV008-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
PAV008-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
PAV008-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；

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. Unopened kit should be stored at 2°C -8°C upon receiving.

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℃-25℃) 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 sterile water 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℃. PAV008-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 1 µg. PAV008-C03 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 0.3 µg. PAV008-C04 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

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ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
PAV008-C02	Anti-AAV8 Antibody Standard	2.5 μg	50 μg/mL	50 μL water
PAV008-C03	Biotin-AAV8 Capsid Protein	1 μg	20 μg/mL	50 μL water
PAV008-C04	Streptavidin-HRP	10 μg	100 μg/mL	100 μL water

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-AAV8 Capsid Protein working fluid:

Dilute Biotin-Anti- AAV8 Capsid Protein to 0.05 μg/mL with Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP to 0.1 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 Anti-AAV8 Antibody as a Standard curve with Dilution Buffer as recommended in Figure 1.

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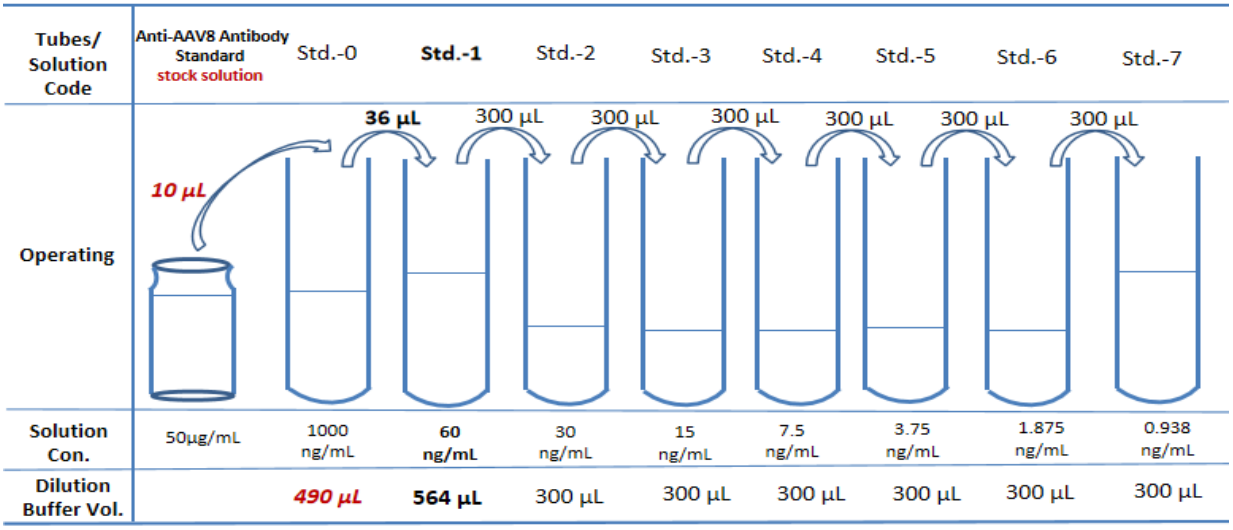
FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-AAV8 Antibody



3. Add Samples

Add 100µL serially diluted Anti-AAV8 Antibody 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 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 Biotin-AAV8 Capsid Protein

For all wells, add 100 µL **Biotin-AAV8 Capsid Protein (dilute to 0.05 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 µL **Streptavidin-HRP (dilute to 0.1 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, avoid light.

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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 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: $R^2 \geq 0.9900$, detection range: 0.938-60 ng/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℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.

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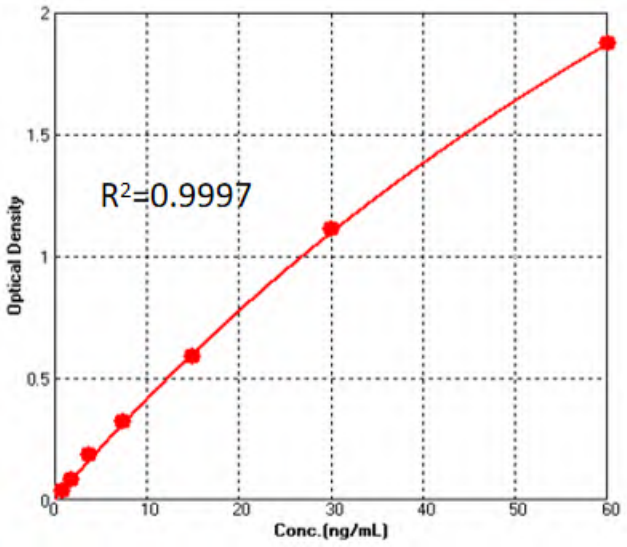
5. The kit should be stored at 2°C to 8°C.

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.

Anti-AAV8 Antibody Standard(ng/mL)	OD450-630nm	OD450-630nm-Blank
60	1.894	1.871
30	1.131	1.108
15	0.611	0.588
7.5	0.342	0.319
3.75	0.206	0.183
1.875	0.106	0.083
0.938	0.060	0.037
Blank	0.023	0.000



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