PAV02-EN.01

Anti-AAV2 Antibody ELISA Kit

Pack Size: 96 tests

Catalog Number： PAV-A002

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-AAV2 Antibody ELISA Kit is developed for the detection of anti-AAV2 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

Adeno-associated virus (AAV) has become one of the most important gene vectors in the field of gene therapy due to its long-term expression, low toxicity, low immunogenicity, and high tissue specificity. Most successful AAV gene therapies for preclinical and clinical studies are limited to natural serotypes, but the presence of neutralizing antibodies against AAV remains a significant barrier to systemic delivery.

This assay kit is used to measure the levels of anti-AAV2 antibodies by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with AAV2 Capsid Protein. First add the standard samples provided in the kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-AAV2 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-AAV2 antibodies present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of anti-AAV2 antibodies bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
PAV002-C01	Pre-coated AAV2 Capsid Protein Microplate	1 plate	Solid	2-8°C	2-8°C
PAV002-C02	Anti-AAV2 Antibody Standard	2.5 μg	Powder	2-8°C	-70°C
PAV002-C03	Biotin-AAV2 Capsid Protein	10 μg	Powder	2-8°C, avoid light	-70°C, avoid light
PAV002-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
PAV002-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C

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PAV002-C06	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
PAV002-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
PAV002-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

Note: *It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

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. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and 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℃. PAV002-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 1μg. PAV002-C03 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 2μg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
PAV002-C02	Anti-AAV2 Antibody Standard	2.5 μg	50 μg/mL	50 μL water
PAV002-C03	Biotin-AAV2 Capsid Protein	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-AAV2 Capsid Protein working fluid:

Dilute Biotin-AAV2 Capsid Protein to 0.2 μg/mL with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×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-AAV2 Antibody as a Standard curve with Dilution Buffer as recommended in Figure 1.

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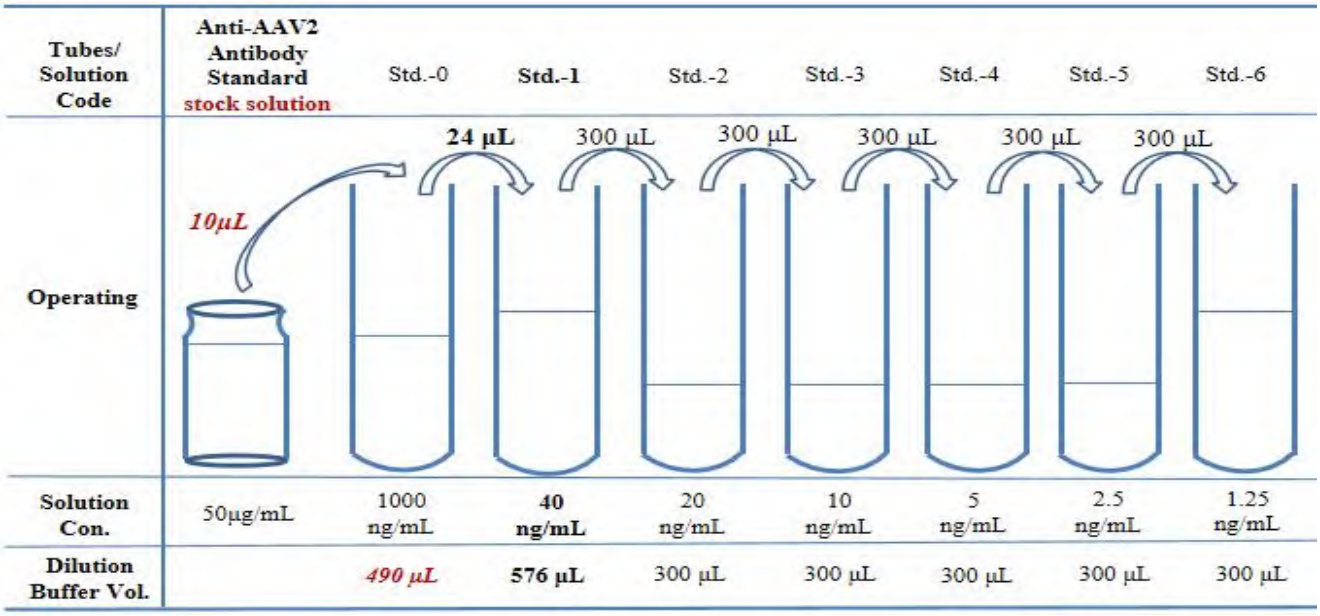
FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-AAV2 Antibody



3. Add Samples

Add 100μL serially diluted **Anti-AAV2 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 1.0 hour.

Note: *It is recommended to set double holes for samples and standard curves to be tested.*

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, soak for 30s, 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-AAV2 Capsid Protein

For all wells, add 100 μL **Biotin-AAV2 Capsid Protein (dilute to 0.2 μg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 hour.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

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For all wells, add 100 μL **Streptavidin-HRP (dilute at 1:2000)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

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 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 within 5 minutes.

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: 1.25-40 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 to 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. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer

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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

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Anti-AAV2 Antibody Standard (ng/mL)	OD450-630nm	OD450-630nm-Blank
40	2.358	2.317
20	1.387	1.346
10	0.760	0.720
5	0.467	0.426
2.5	0.306	0.266
1.25	0.148	0.108
Blank	0.040	0.000

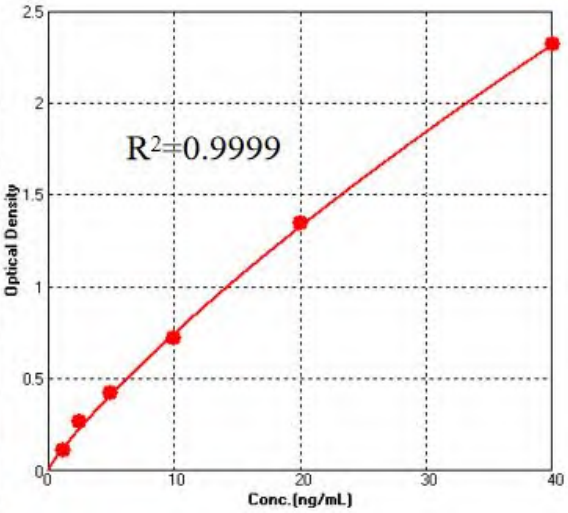
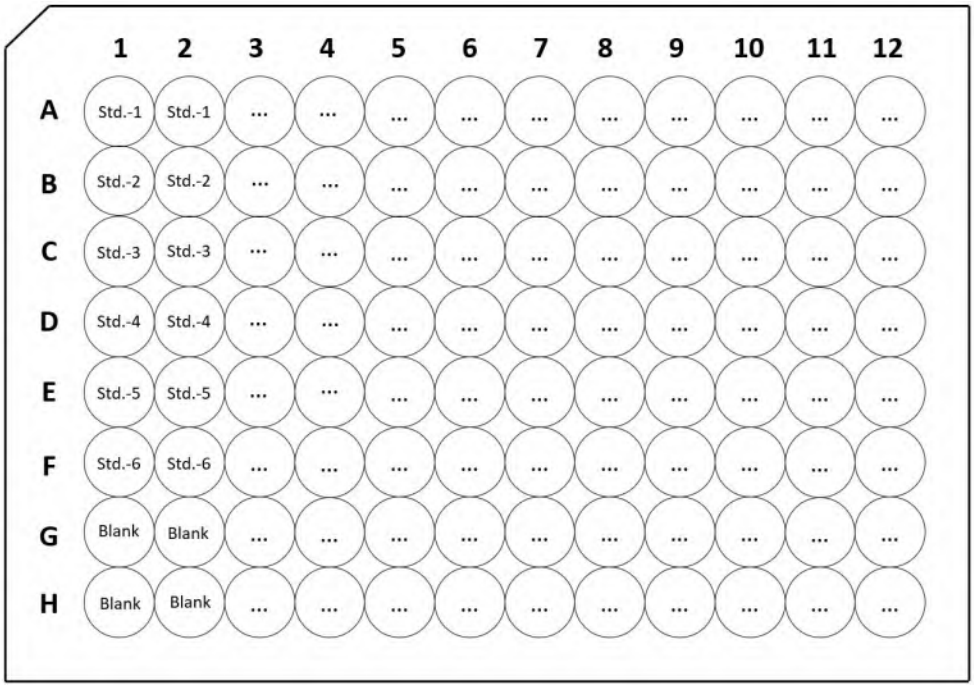


PLATE LAYOUT



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Note: *Blank is a Blank Dilution Buffer hole.*

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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