



AAVX01-EN.01

AAV Capsid Titration Detection Antibody Pair (Universal)

Catalog Number: AAVX-01

Pack Size: 960 tests

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

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

INTENDED USE

This pair is developed for the detection and quantitative determination of AAV capsid titration in AAV gene therapy product preparation processing. This pair recognizes AAV1, AAV2, AAV3, AAV5 and AAV8, not recognized AAV9. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This assay Pair is used to measure the levels of AAV capsid by employing a standard sandwich-ELISA format. Firstly, attach the AAV capsid Capture Antibody to the microplate, add your samples to the plate, incubate and wash the wells. Then add the AAV Capsid Detection Antibody to the plate, incubate and wash the wells. Next add HRP-Conjugated Antibody to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. 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 AAV capsid bound.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (960tests)	Format	Storage	
				Unopened	Opened
AAVX01-C01	AAV Capsid Capture Antibody	120 µg	Powder	-20°C ~ -70°C	-70°C
AAVX01-C02	AAV Capsid Detection Antibody	60 µg	Powder	-20°C ~ -70°C	-70°C
AAVX01-C03	HRP-Conjugated Antibody	15 µg	Powder	-20°C ~ -70°C	-70°C

STORAGE

1. The unopened product is stable for 36 months from the date of manufacture if stored at -20°C to -70°C.
2. The opened product should be stored per Table 1. The shelf life is 90 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.

OTHER MATERIALS & SOLUTIONS REQUIRED

- 1.96 well microplates: Corning , Catalog# 42592
- 2.Coating Buffer (1xCBS): 0.015mol/L Na₂CO₃, 0.035mol/L NaHCO₃, 0.0077mol/L NaN₃, pH9.59
- 3.1xWashing Buffer(1xPBST): 0.05% Tween-20 in TBS, pH7.4
- 4.Blocking Buffer: 2% BSA in 1xWashing Buffer
- 5.Dilution Buffer: 0.5% BSA in 1xWashing Buffer
- 6.Substrate Solution: InnoReagents、 Catalog # TMB-S-004
- 7.Stop Solution: 2N H₂SO₄

Note: The kit does not contain different AAV serotypes and needs to be prepared by the user.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20°C-25°C) before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoid vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 3 times, and the size of the aliquot should not be less than 10 µg.

Table 2. Preparation method

ID	Components	Size (960tests)	Storage solution concentration.	Reconstituted water Vol.
AAVX01-C01	AAV Capsid Capture Antibody	120 µg	400 µg/mL	300 µL
AAVX01-C02	AAV Capsid Detection Antibody	60 µg	400 µg/mL	150 µL
AAVX01-C03	HRP-Conjugated Antibody	15 µg	100 µg/mL	150 µL

RECOMMENDED SAMPLE PREPARATION

1. Coating

Dilute AAV Capsid Capture Antibody (AAVX01-C01) stock solution (400 µg/mL) to 1.0 µg/mL with Coating Buffer to make AAV Capsid Capture Antibody working solution.

Add 100 µL of AAV Capsid Capture Antibody working solution (1.0 µg/mL) to each well, seal the plate

with microplate sealing film and incubate overnight (or 16 hours) at 4°C.

2. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, 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.

3. Blocking

Add 300 µL Blocking Buffer to each well, seal the plate with microplate sealing film and incubate at room temperature for 2.0 hours.

4. Washing

Repeat step 2.

5. Add Samples

Add 100 µL Samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

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

6. Incubation

Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

7. Washing

Repeat step 2.

8. Add AAV Capsid Detection Antibody

Dilute AAV Capsid Detection Antibody (AAVX01-C02) stock solution (400 µg/mL) to 0.5 µg/mL with Dilution Buffer to make AAV Capsid Detection Antibody working solution.

For all wells, add 100 µL AAV Capsid Detection Antibody (0.5 µg/mL) working solution. Please prepare it for one-time use only.

9. Incubation

Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

10. Washing

Repeat step 2.

11. Add HRP-Conjugated Antibody

For all wells, add 100 μ L HRP-Conjugated Antibody (AAVX01-C03) (dilute to 0.08 μ g/mL) working solution. Please prepare it for one-time use only, avoid light.

12. Incubation

Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

13. Washing

Repeat step 2.

14. Substrate Reaction

Add 100 μ L Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

15. Termination

Add 50 μ L 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.

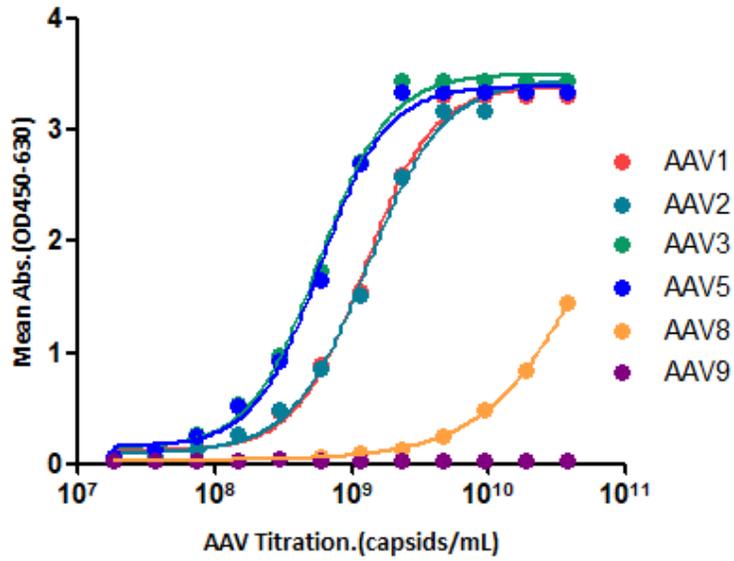
16. Data Recording

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 OD450nm with the value read at OD630 nm.

TYPICAL DATA

This data is for reference only, users need to do their own method development according to the actual situation.



Immobilized AAV Capsid Capture Antibody (AAVX01-C01) at 1.0 µg/mL (100 µL/well) can bind AAV Capsid, and then add AAV Capsid Detection Antibody (AAVX01-C02) at 0.5 µg/mL (100 µL/well), final detection was performed using HRP-Conjugated Antibody (AAVX01-C03)。