KACTUS

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AAV2 Titration ELISA Kit

Catalog # AV2-MM00B (96 T)

Component Name	Size	Component Description	Storage
AAV2 Standard (AV2-MM00B-1)	3 vials (6.6E+09 capsids/mL)	Lyophilized AAV2 powder	2-8°C
20X Detection Antibody (AV2-MM00B-2)	1 x 750µL	Biotinylated anti-AAV2 antibody which binds to AAV2 in standard and test samples.	
Pre-coated plate (AV2-MM00B-3)	1 x 96-well plate	Microtiter plate coated with monoclonal anti-AAV2 antibody.	
HRP Conjugate (AV2-MM00B-4)	1 x 15mL	Streptavidin Peroxidase conjugate that binds to detection antibody and catalyzes color development.	
20X Assay Buffer (AV2-MM00B-5)	2 x 30mL	For diluting standards, samples and washing plate. Dilute to 1X before use.	
TMB (AV2-MM00B-6)	1 x 15mL	Chromogenic substrate for HRP.	
Stop Solution (AV2-MM00B-7)	1 x 10mL	0.5M H_2SO_4	

OTHER EQUIPMENT REQUIRED BUT NOT PROVIDED:

- Microplate reader (full wavelength or with 450nm filter)
- Plate washer

ASSAY PERFORMANCE

Detection Range: 1.03E+08 capsids/mL - 6.60E+09 capsids/mL
Sensitivity: 5E+07 capsids/mL
Precision: CV < 5% indicates high precision. CV should be < 10% for standards and samples. Retest any samples with CV ≥ 10%.

STORAGE CONDITIONS AND EXPIRATION

Store kit components at 2-8°C. The kit is valid for 12 months from the production date. Reconstituted AAV2 standard stable at 2-8°C for 2 weeks. -20°C or below for long-term storage and avoid repeated freeze-thaw cycles.

APPLICATIONS

- Intact AAV2 Wild Type Virions
- AAV2 Recombinant Virions
- Assembled AAV2 Virions
- Intact Empty AAV2 Capsids

DETECTION PRINCIPLE

This kit uses sandwich ELISA to determine the titer of AAV2 capsids in the test sample. The capture AAV2 monoclonal antibody is pre-coated and treated on a 96-well reaction plate. AAV2 standard or sample is added to the pre-coated plate and specifically binds to the capture antibody. The biotinylated detection AAV2 antibody is then added to the plate to bind the immune complex. Next, the streptavidin HRP conjugate is added to react with the biotin molecules. The addition of TMB results in color changes and the amplitude of the color change is proportional to the amount of AAV2 capsids that specifically bind to the plate. The reaction is stopped with the addition of stop solution and the absorbance is measured at 450nm. The sample AAV2 capsid titer is calculated from the AAV2 standards titration curve.

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OPERATING PROCEDURES

Equilibrate the kit to room temperature before use.

Reagent Preparation:

1. Prepare 1X Assay Buffer (Dilute 20X Assay Buffer with distilled H_2O)
2. Prepare 1X Detection Antibody (Dilute the detection antibody (20X) with 1X Assay Buffer)

Sample and Standard Preparation:

3. **Preparation of AAV2 capsid standards**: Add 700µL of distilled H_2O to one vial of standard. Dissolve at room temperature for 10-20 min, mix gently and avoid vortexing. The titer of the reconstituted standard is 6.60E+09 capsids/mL. Two-fold serial dilution of the AAV2 standard with 1X Assay Buffer for the titration curve in duplicates is recommended.

Standard	Concentration (capsids/mL)	Final Dilution
A	6.60E+09	Undiluted
B	3.30E+09	1:2
C	1.65E+09	1:4
D	8.25E+08	1:8
E	4.13E+08	1:16
F	2.06E+08	1:32
G	1.03E+08	1:64
H (1X Assay Buffer)	0	N/A

4. **Sample preparation**: Dilute the sample with 1X Assay Buffer to make the capsid titer quantification fall within the linear range.

ELISA

5. **Equilibration**: Take out the 96-well plate, seal unused strips and immediately put them back at 2-8°C. Wash the plate with 1X Assay buffer. Pat dry.
6. **Incubation**: Add the standard and samples to the 96-well plate (100 µL per well). Incubate at 37°C shaker (600 rpm) for **60 min**.
7. **Adding detection antibody**: Wash the 96-well plate with 300 µL 1X Assay buffer 3 times. Pat dry and immediately add 1X detection antibody (100 µL per well). Incubate at 37°C shaker (600 rpm) for **60 min**.
8. **Adding Streptavidin-HRP conjugate**: Wash the 96-well plate with 300 µL 1X Assay buffer 3 times. Pat dry and add streptavidin-HRP conjugate (100 µL per well). Incubate at 37°C shaker (600 rpm) for **60 min**.
9. **Adding TMB Substrate**: It is recommended to preheat the TMB Substrate to 37°C. Wash the 96-well plate with 300 µL 1X Assay buffer 4 times. Pat dry and add 100 µL of TMB Substrate to each well. Incubate at 37°C in the darkness for **10 min**.
10. **Adding Stop Solution**: Add 50 µL of Stop Solution to each well and mix gently. Immediately read the OD value of each well at 450nm by a microplate reader. It is recommended to read the OD450 values within 5 min after adding the Stop Solution.

DATA ANALYSIS

1. Create a standard curve by plotting the standard concentrations on the x-axis and the OD450 values on the y-axis of a scatterplot. If standards were run in duplicate or triplicate, use the average value. We recommended fitting the data with a 4-parameter logistic fit curve to use as the standard curve. However, other methods such as linear and logarithmic methods can obtain better fitting results, depending on the specific experimental needs.
2. Calculate the sample titer by entering the sample OD450 value into the equation for the standard curve. If you dilute the sample, multiply by the dilution factor. The lower limit of quantitation (LOQ) is 1.06E+08capsids/mL. If the OD450 of the sample falls above the valid linear range, the sample should be further diluted and retested.

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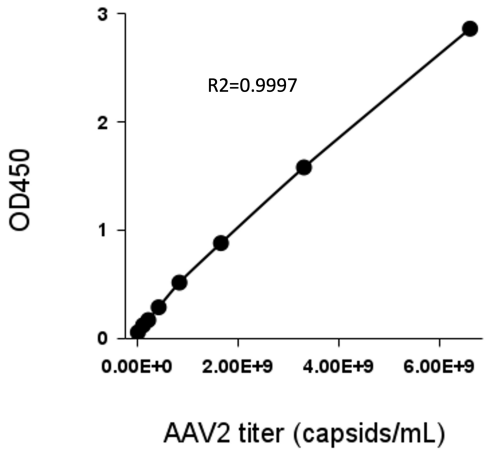
EXAMPLE DATA

AAV2 titer (capsids/mL)	OD450
6.60E+09	2.86
3.30E+09	1.58
1.65E+09	0.881
8.25E+08	0.517
4.13E+08	0.289
2.06E+08	0.171
1.03E+08	0.123
0	0.058



PRECAUTIONS

1. The microplate has detachable strips. Do not touch the bottom of the well while disassembling.
2. Do not leave the plate too long after each wash to avoid the plates drying out.
3. 20X Assay Buffer may precipitate at 4°C due to high salt concentration. The precipitates can be redissolved at room temperature.
4. Do not use this kit with components from other commercial kits, and do not mix components from different batches of kits. A standard curve must be prepared for each plate, and duplicates are recommended.
5. All reagents must be equilibrated to room temperature (18-25°C) before use. The TMB substrate should be warmed to 37°C before use.
6. Make sure there is no liquid left in each well after each wash.
7. Using a plate washer can reduce the experimental error. For the manual wash it is recommended to soak the plate in 1X Assay Buffer for 1 minute after each addition.
8. The TMB substrate incubation needs to be protected from light and strictly controlled within 10 minutes.
9. The Stop Solution contains sulfuric acid and may cause skin or eye burns. Rinse immediately with plenty of water and seek medical assistance if necessary.
10. When dissolving standard mix gently and avoid vortexing.
11. This product is for scientific research only and may not be used for other purposes.