ABIN7539378

Sandwich ELISA Kit for Adeno-Associated Virus 2

Adeno-Associated Virus 2 (AAV-2)

96 tests

For research use only
Not for use in clinical diagnostic procedures
Version Oct 2024

Intended use

This kit uses sandwich ELISA to determine the titer of AAV capsids in the test sample.

Reagents and materials provided

- Pre-coated 96-well plate
- Reference Standard
- Detection Antibody (20x)
- HRP Conjugate
- Assay Buffer (20x)
- TMB Substrate
- Stop Solution
- Instruction manual

Materials required but not supplied

- Microplate reader (full wavelength or with 450nm filter)
- Plate washer
- Temperature-controlled plate shaker
- Pipettes

Storage of the kit

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

Note:

It is strongly recommended to use the remaining reagents within 1 month, if this is done before the expiry date of the kit. Please refer to the label on the kit packaging for the expiration date of the kit. All components are stable until the expiration date.

Sample collection and storage

Sample type	Collection procedure
Cell Culture Supernatant	Centrifuge samples for 20 min at 1000×g at 2-8 °C. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles
Cell Lysates	Cells need to be lysed before assaying according to the following directions. Gently wash adherent cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g (suspension cells can be collected by centrifugation directly). Wash cells 3 times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of 10^7cells/mL. If it is necessary, the cells could be subjected to ultra sonication till the solution is clarified. Centrifuge for 10min at 1500×g at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20 °C (≤ 1 month) or -80 °C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.

Reagent preparation

1. Prepare 1X Assay Buffer (Dilute 20X Assay Buffer with distilled H2O)
2. Prepare 1X Detection Antibody (Dilute the detection antibody (20X) with 1X Assay Buffer)
3. Preparation of AAV capsid standards: Add 700µL of distilled H2O to one vial of standard. Dissolve at room temperature for 10-20 min, mix gently and avoid vortexing. Two-fold serial dilution of the AAV8 standard with 1X Assay Buffer for the titration curve in duplicates is recommended.
4. Sample preparation: Dilute the sample with 1X Assay Buffer to make the capsid titer quantification fall within the linear range.

Sample preparation

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

Assay procedure

1. Equilibration: Take out the 96-well plate, seal unused strips and immediately put back at 2-8°C. Wash the plate with 1X Assay buffer. Pat dry.
2. Incubation: Add the standard and samples to the 96-well plate (100 µL per well). Incubate at 37°C for 60 min.
3. 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 for 60 min.
4. 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 for 60 min.
5. 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.
6. 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.

Test principle

The capture AAV monoclonal antibody is pre-coated and treated on a 96-well reaction plate. AAV standard or sample is added to the pre-coated plate and specifically binds to the capture antibody. The biotinylated detection AAV 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 AAV 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 AAV capsid titer is calculated from the AAV standards titration curve.

Calculation of results

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 diluted the sample, multiply by the dilution factor. If the OD450 of the sample falls above the valid linear range, the sample should be further diluted and retested.

Typical data

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).

Detection range

The detection range of the kit is 103000000 capsids/mL – 6600000000 capsids/mL
The standard curve concentrations used for the ELISA's were 6600000000 capsids/mL, 3300000000 capsids/mL, 1650000000 capsids/mL, 825000000 capsids/mL, 413000000 capsids/mL, 206000000 capsids/mL, 103000000 capsids/mL

Sensitivity

The minimum detectable dose of AAV2 is typically less than 50000000 capsids/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.
It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Note:
Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between AAV2 and all the analogues, therefore, cross reaction may still exist.

Precision

Retest any samples with CV > 10%.

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % prior to the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored. It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Assay procedure summary

1. Prepare all reagents, samples and standards,
2. Wash plate with 1X Assay buffer
3. Add 100µL standard or sample to each well. Incubate 1 hour at 37 °C,
4. Aspirate and wash with 300 µL 1x Assay buffer 3 times,
5. Add 100µL Detection Antibody. Incubate 1 hour at 37 °C,
6. Aspirate and wash with 300 µL 1x Assay buffer 3 times,
7. Add 100µL Streptavidin-HRP conjugate. Incubate 1 hour at 37 °C,
8. Aspirate and wash with 300 µL 1x Assay buffer 4 times,
9. Add 100 µL TMB Substrate. Incubate 10 minutes in the dark at 37 °C,

10. Add 50μL Stop Solution. Read at 450nm immediately.

Important note

1. The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.
2. Limited by the current conditions and scientific technology, we cannot perform a complete identification and analysis of the raw material used. Therefore, the use of the kit may be associated with some qualitative and technical risks.
3. We are only responsible for the kit itself, not for the samples used in the test. The possible amount of sample used in the whole test should be calculated in advance and sufficient sample material should be provided.
4. Each kit undergoes a very strict QC testing. Nevertheless, end-user results may differ from our internal results due to unexpected transport conditions or different laboratory equipment. Intra-assay deviations between kits from different lots can also be related to this.
5. The test results depend on the validity of the products, so the kit should be used before the expiration date and stored according to the instructions.
6. Even the same user may obtain different results in two separate experiments. To obtain reproducible results, each step of the assay should be controlled.
7. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
8. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.
9. Protect all reagents from strong light during storage and incubation. All reagent bottle caps should be tightly closed to prevent evaporation and contamination with microorganisms. The TMB substrate should remain colourless until it reacts with the enzyme.
10. A freshly opened ELISA plate may show a water-like substance, which is normal and does not affect the test results. Return unused wells to the foil pouch and store as before.
11. Mistakes in reagent preparation and application, as well as incorrect parameter setting for the plate reader, can lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at a wavelength of 450 ± 10 nm is suitable for absorbance measurement. Please read the instructions carefully and set

up the instrument before the experiment.

12. Do not reuse the reconstituted standard and the prepared working solutions. The unused stock solutions should be stored according to the storage conditions.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Troubleshooting

Problem	Possible Source	Corrective Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate and substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested	Read within the time recommended in

Problem	Possible Source	Corrective Action
	reading time	the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Resample and repeat assay

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