AAV5 Titration ELISA

Enzyme-linked Immunosorbent Assay for the Quantitative Determination of AAV Serotype 5 Particles in Cell Culture Supernatants and Purified Virus Preparations

Art. No.: PRAAV5
Contents: 12 × 8 Determinations
Storage: 2-8°C

For research use only!

1. Introduction
Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses, which are a subject of intense studies as viral vectors for gene therapy. The virus transduces a wide variety of dividing and non-dividing cells showing long-term gene expression with low cellular immune response. AAV has been used in several clinical trials (e.g. FIX, CFTR, Parkinson’s disease, Canavan disease) showing no serious vector-related adverse effects. Methods for the characterization of AAV preparations currently include titration ELISA, real-time PCR, DNA dot blot, determination of transducing units, infectious center assay, SDS-PAGE or electron microscopy. Immunotitratation by PROGEN’s AAV5 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV5 wt virions, AAV5 recombinant virions or assembled and intact empty AAV5 capsids.

2. Test Principle
The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV5 capsids is coated onto microtiter strips and is used to capture AAV5 particles from the specimen. Captured AAV particles are detected in two steps. First a biotin-conjugated monoclonal antibody to AAV5 is bound to the immune complex. Second, a streptavidin peroxidase conjugate reacts with the biotin molecules. Addition of substrate solution results in a color reaction, which is proportional to the amount of specifically bound viral particles. The absorbance is measured photometrically at 450 nm.

The Kit Control provided contains an AAV5 particle preparation of empty capsids. Two-fold serial dilutions of the material result in a typical titration curve. The curve allows the quantitative determination of samples of an unknown particle titer.

3. Material Required
Precision pipettes
Sterile pipette tips
Distilled water
Test tubes for specimen dilutions
Incubator for 37°C
ELISA Reader (450 nm)

4. Contents of Test Kit
MTP Microtiter Plate, 12 × 8-well-strips, coated with mouse monoclonal antibody to AAV5 in resealable aluminum bag with desiccant. Ready-to-use.
KC Kit Control (AAV5), lyophilized, 3 vials. Reconstitute before use.
ASSB 20x Assay Buffer 20x, 3 x 20 mL. Dilute before use.
B CON Anti-AAV5 Biotin Conjugate, lyophilized. Reconstitute and dilute before use.
CON 20x Streptavidin Peroxidase Conjugate 20x, 750 µL. Dilute before use.
S Substrate, TMB (tetramethylbenzidine), 12 mL. Ready-to-use.
STOP Stop Solution, 13 mL. Ready-to-use.
Adhesion foil
All fluid components except S and STOP contain a preservative!

5. Preparation of Reagents
Prior to use, allow kit to reach room temperature (RT, 20-26°C). Buffer concentrates may contain salt crystals which dissolve quickly at 37°C. Let buffer reach room temperature before use.
Unused strips should be stored at 2-8°C in the resealable aluminum bag with desiccant.
Dilute required volumes of reagents immediately before use!

Predilutions of components:
Assay buffer: Dilute 1:20 with distilled water for diluted Assay Buffer.
Kit Control: Reconstitute with 500 µL distilled water to obtain a defined amount of particles/mL (see label for exact concentration). Mix carefully (do not vortex) before use.

Anti-AAV5 Biotin Conjugate: Reconstitute with 750 µL distilled water. Immediately before use dilute 1:30 with diluted Assay Buffer for diluted AAV5 Biotin Conjugate.

Streptavidin Peroxidase Conjugate: Immediately before use dilute 1:20 with diluted Assay Buffer for diluted Streptavidin Peroxidase Conjugate.

6. Stability of Reagents

Store test kit and components at 2-8°C. The unopened reagents are stable until the indicated expiry date at 2-8°C.

Stability after opening:
4 weeks at 2-8°C

7. Kit Control and Specimen Dilution

Please consider the following documents provided with the kit:
1. Lot-specific Reference Curve
2. Quality Control Certificate

Dilute the reconstituted Kit Control (KC) in diluted Assay Buffer in steps of 1:2; e.g.:

Undiluted ⇒ 1:2 ⇒ 1:4 ⇒ 1:8 ⇒ 1:16 ⇒ 1:32 ⇒ 1:64

For orientation, please find an example of dilutions and corresponding titer of the Kit Control on the provided lot-specific Reference Curve.

Pre-dilute your specimen containing AAV5 particles to reach a concentration within the linear range of the ELISA using diluted Assay Buffer. For orientation, please find the range of the ELISA on the lot-specific Reference Curve. Then, dilute your specimen in steps of 1:2 in diluted Assay Buffer. A minimum of 2-3 different dilutions should be tested.

8. Test Procedure

1. Pipette duplicates of 100 µL of diluted Assay Buffer (Blank), serial dilutions of Kit Control and specimen (both in diluted Assay Buffer) into each of the corresponding wells of the microtiter strips. Seal strips with adhesion foil and incubate for 1 h at 37°C.

2. Empty content of microtiter strips. Fill each well with 200 µL of diluted Assay Buffer, incubate approximately 5 sec, empty and tap inverted plate onto absorbent paper. Repeat washing step 2×.

3. Pipette 100 µL per well of diluted Biotin Conjugate. Seal strips with adhesion foil and incubate for 1 h at 37°C.

4. Repeat washing step as described in 2.

5. Pipette 100 µL per well of diluted Streptavidin Conjugate into each well. Seal strips with adhesion foil and incubate for 1 h at 37°C.

6. Repeat washing step as described in 2.

7. Pipette 100 µL of ready-to-use Substrate into each well. Incubate for 15 min at RT.

8. Stop color reaction by adding 100 µL of ready-to-use Stop Solution into each well.

9. Within 30 min, measure intensity of color reaction with a photometer at 450 nm wavelength (optional: reference wavelength 650 nm).

9. Calculation of Results

Calculate the average absorbance values for each duplicate set Kit Control dilutions and sample dilutions.

Create a standard curve by plotting the mean absorbance value of each Kit Control dilution (y-axis) against the corresponding concentration (x-axis).

Use a best fit curve for calculating the results. We suggest using a suitable computer program for the calculation. A 4-parameter logistic fit (4PL) is recommended. Calculate the particle titer of your specimens.

10. Test Validity

The absorbance value of the undiluted Kit Control should be > 1.2.

The absorbance value of the Blank should be < 0.4.

11. Notes for the User

Release notes

The instruction manual is only valid in combination with the lot-specific documents (→ Reference Curve and Quality Control Certificate), which are enclosed in each kit. Please make sure to use the instruction manual in the version number corresponding to the Lot number!

Security notes

All fluid components except Substrate and Stop Solution contain a preservative! Do not swallow! Avoid any contact with skin or mucous epithelia!
A Safety data sheet is available on request!
Stop Solution (sulphuric acid) and components of substrate may cause skin irritations. In the event of acid or substrate coming into contact with eyes, rinse out immediately with plenty of water and consult a physician!

Safety data sheet is available on request!

Disposal considerations

Product: Chemicals and biological materials must be disposed of in compliance with the respective national regulations.

Packaging: Packaging must be disposed of in compliance with the country-specific regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled.

Measures after damage on transport

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerable damaged components for a test procedure. Such components or kits should be stored at 2-8°C until the complaint is handled.

12. Literature


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Short Description AAV5 Titration ELISA

**Preparation of Reagents**

**Assay Buffer (ASSB 20x)**
1:20 with distilled water

**Kit Control and Specimen Dilution**
(Kit Control reconstituted in 500 µL distilled water)

in steps of 1:2
with diluted Assay Buffer

**Anti-AAV5 Biotin Conjugate (B CON)**
(reconstituted in 750 µL distilled water)
1:30 immediately before use, with diluted Assay Buffer

**Streptavidin Peroxidase Conjugate (CON 20x)**
immediately before use, with diluted Assay Buffer

**Blank, Kit Control Dilutions, Sample Dilutions**
100 µL

Incubate 60 min at 37°C
Wash 3 x with 200 µL diluted Assay Buffer

**Diluted Anti-AAV5 Biotin Conjugate**
100 µL

Incubate 60 min at 37°C
Wash 3 x with 200 µL diluted Assay Buffer

**Diluted Streptavidin Peroxidase Conjugate**
100 µL

Incubate 60 min at 37°C
Wash 3 x with 200 µL diluted Assay Buffer

**Substrate**
100 µL

Incubate 15 min at room temperature

**Stop Solution**
100 µL

Read absorbance at 450 nm within 30 min
(optional: reference wavelength 650 nm)