AAV6 Titration ELISA

Enzyme-linked Immunosorbant Assay (ELISA) for the Quantitative Determination of AAV Serotype 6 Particles in Cell Culture Supernatants and Purified Virus Preparations

Art. No.: **PRAAV6**

Contents: **12 x 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 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, 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.
Immunotitration by PROGEN's AAV6 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV6 wt virions, AAV6 recombinant virions or assembled and intact empty AAV6 capsids.

2. Test Principle

The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV6 capsids (ADK6) is coated onto strips of a microtiter plate and is used to capture AAV6 particles from the specimen. Captured AAV particles are detected in two steps. First, a biotin-conjugated monoclonal antibody to AAV6 (ADK6) 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 provided Kit Control contains an AAV6 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 x 8-well-strips, coated with mouse monoclonal antibody to AAV6 in resealable aluminum bag with desiccant. Ready- to-use.
KC	Kit Control (AAV6), lyophilized, 3 vials. Reconstitute before use.
ASSB 20x	Assay Buffer 20x, 3 x 20 mL. Dilute before use.
B CON	Anti-AAV6 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!

***Predilution of components*:**

ASSB 20x (Assay Buffer): Dilute **1:20** with distilled water for diluted Assay Buffer.

KC (Kit Control): Reconstitute with **500 µL** distilled water to obtain a defined amount of particles/mL (see label for exact concentration). Mix carefully (don't vortex) before use.

B CON (Anti-AAV6 Biotin Conjugate): Reconstitute with **750 µL** distilled water. Immediately before use, dilute **1:20** with diluted Assay Buffer for diluted AAV6 Biotin Conjugate.

CON 20x (Streptavidin Peroxidase Conjugate): Immediately before use, dilute **1:20** with diluted Assay Buffer for diluted Streptavidin Peroxidase Conjugate.

6. Stability of Reagents

Store the 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 $\overset{1:2}{\Rightarrow}$ 1:2 $\overset{1:2}{\Rightarrow}$ 1:4 $\overset{1:2}{\Rightarrow}$ 1:8 $\overset{1:2}{\Rightarrow}$ 1:16 $\overset{1:2}{\Rightarrow}$ 1:32 $\overset{1:2}{\Rightarrow}$ 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 AAV6 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 2x.
3. Pipette 100 µL of diluted Biotin Conjugate into each well. 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 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 a wavelength of 450 nm.
   (optional: reference wavelength 650 nm)

9. Calculation of Results

Calculate the average absorbance values for each duplicate set of 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 < 0.3.

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!

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!

A 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 may be recycled.

Measures after damage on transport

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

12. Reference

Sonntag F, Köther K, Schmidt K, Weghofer M, Raupp C, Nieto K, Kuck A, Gerlach B, Böttcher B, Müller OJ, Lux K, Hörer M, Kleinschmidt JA. The Assembly-Activating Protein Promotes Capsid Assembly of Different Adeno-Associated Virus Serotypes. *J Virol* (2011) 85(23):12686-12697. DOI: 10.1128/JVI.05359-11. PMC: 3209379.

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Short Description AAV6 Titration ELISA

Preparation of Reagents, Kit Control and Specimen

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

Kit Control (KC) and Specimen Dilution
(Kit Control reconstituted in 500 µL distilled water) **in steps of 1:2**
with diluted Assay Buffer

Anti-AAV6 Biotin Conjugate (B CON)
(reconstituted in 750 µL distilled water) **1:20**
immediately before use, with diluted Assay Buffer

Streptavidin Peroxidase Conjugate (CON 20x) **1:20**
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-AAV6 Biotin Conjugate	**100 µL**

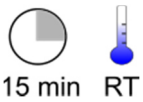
Incubate 60 min at 37°C
Wash 3 x with 200 µL diluted Assay Buffer

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)