PROGEN Biotechnik GmbH

AAV-8 Titration

ELISA

Enzyme Immunoassay for the Quantitative Determination of AAV Serotype 8 Particles in Cell Culture Supernatants and Purified Virus Preparations

Art. No.: **PRAAV8**
Content: **12 × 8 Determinations**
Storage: **2-8°C**

For research use only!

1. Introduction

Adeno-associated virus (AAV) is a non pathogenic ssDNA virus that is a topic of intense study in gene therapy. The virus transduces a wide variety of dividing and non-dividing cells showing long-term gene expression with no cellular immune response. AAV has been used in several clinical trials (e.g. FIX, CFTR, Parkinsons's, Canavan disease) showing no serious vector-related adverse effects. Methods for the characterisation 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.

2. Test Principle

The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV-8 capsids (ADK8) is coated onto microtiter strips and is used to capture AAV-8 particles from the specimen. Captured AAV particles are detected in two steps. First a biotin-conjugated monoclonal antibody to AAV-8 (ADK8) is bound to the immune complex. In the second step 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 AAV 2/8 particle preparation, including empty capsids. It shows a typical titration curve when used in dilutions of steps of two (Fig. 1). It allows the quantitative determination of samples of an unknown particle titer (immunological titer) and the calibration of an inhouse AAV8 preparation (e.g. infectious titer, transducing units, DNA dot blot titer).

3. Material Required

Precision pipettes
Sterile pipette tips
Distilled water
Vials for specimen dilutions
Incubator for 37°C
Microtiter plate spectrophotometer (450 nm)

4. Contents of Test Kit

MTP	Microtiter Plate, 12 × 8-well-strips, coated with mouse monoclonal antibody to AAV-8 in resealable aluminum bag with desiccant. Ready-to-use.
STD	Standard (AAV 2/8 WL 217S), lyophilized, 2 vials. Reconstitute before use.
SB 20x	Sample Buffer, 20x, 20 mL. Dilute before use.
WASH 20x	Wash Buffer 20x, 2x 20 mL. Dilute before use.
B 20x	Anti-AAV-8 Biotin Conjugate 20x, lyophilized. Reconstitute and dilute before use.
CON 20x	Streptavidin Peroxidase Conjugate 20x, liquid (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 components except S and STOP contain 0.01% Thimerosal as preservative!

5. Preparation of Reagents

Allow kit to reach room temperature (RT, 20-26°C). Buffer concentrates may contain salt cristals which dissolve quickly at 37°C. Let buffer reach room temperature before use.

Store unused strips in the resealable aluminum bag with desiccant at 2-8°C.

Dilute required volumes of reagents immediately before use!

***Ready-to-use solutions*:**

Sample buffer: Dilute 1:20 with distilled water for ready-to-use sample buffer.

Wash buffer: Dilute 1:20 with distilled water for ready-to-use wash buffer.

Anti-AAV-8 biotin conjugate*: Reconstitute with 750 µL distilled water.

Dilute 1:20 with ready-to-use wash buffer for ready-to-use AAV-8 biotin conjugate.

Streptavidin peroxidase conjugate*: Dilute 1:20 with ready-to-use wash buffer for ready-to-use streptavidin peroxidase conjugate.

Reconstitution of Standard:

Reconstitute with **500 µL** distilled water; contains defined amount of particles/mL (see label for exact concentration).

* Dilute immediately before use.

6. Stability of Reagents

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

Stability after opening:

6 months at 2-8°C:

WASH 20x, SB 20x, CON 20x, S, MTP (in aluminum bag with desiccant)

2 weeks after reconstitution (when stored at 2-8°C):

STD, B 20x

7. Standard and Specimen Dilution

The range of the ELISA covers 1.45×10^7 – 9.24×10^8 particles/mL. Dilute specimen containing AAV-8 particles to reach a concentration within the linear range of the ELISA using ready-to-use sample buffer.

Dilute **specimen** in steps of 1:4. A minimum of 2-3 different dilutions should be tested.

Dilute the reconstituted **Standard (STD)** in ready-to-use sample buffer (see examples for dilution in Figure 1 and Table 1).

Fig. 1: Example of a Titration Curve of Standard

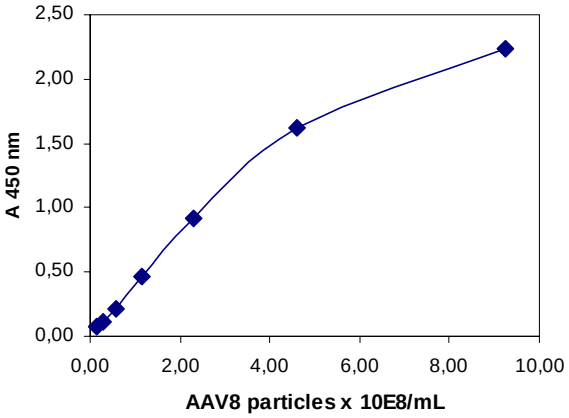

Table 1: Example of Titration of Standard

Standard	Capsids/mL	A 450 nm
Undiluted (1:1)	9.24×10^8	2.230
1:2	4.62×10^8	1.625
1:4	2.31×10^8	0.912
1:8	1.16×10^8	0.469
1:16	5.8×10^7	0.219
1:32	2.9×10^7	0.108
1:64	1.45×10^7	0.080

The values in this table correspond with Figure 1

8. Test Procedure

1. Pipette 100 µL of ready-to-use sample buffer (Blank), serial dilutions of Standard and specimen (both diluted in ready-to-use sample buffer) into the wells of the microtiter strips. Seal strips with adhesion foil provided and incubate for 1 h at 37°C.
2. Empty contents of microtiter strips.

   Fill wells with 200 µL each of ready-to-use wash buffer, incubate approximately 5 sec, empty and tap inverted plate onto absorbant paper. Repeat washing step 2×.
3. Pipette 100 µL per well of ready-to-use 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 ready-to-use streptavidin conjugate. 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 per well of ready-to-use substrate. Incubate for **15 min at RT**.
8. Stop color reaction by adding 100 µL of stop solution into each well.
9. Measure intensity of color reaction with a photometer at 450 nm wavelength within 30 min.

9. Calculation of Results

Create a titration curve by using e.g. semi-logarithmic paper and plotting the OD readings (y-axis) of the serial dilution of the Standard (x-axis) analogously to Fig. 1.

Use this standard curve for the calculation of the particle titer of unknown specimens.

10. Quality Control

Standard (undiluted)	OD > 1.2
Blank	OD < 0.2

11. Notes for the User

Security notes

All components except S and STOP contain 0.01%Thimerosal as preservative! Do not swallow! Avoid any contact with skin or mucous epithelia!

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. Store such components or kits until the complaint is handled.

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Date of release: 17.11.2011