AAV1 Titration ELISA

Enzyme Immunoassay for the Quantitative Determination of AAV Serotype 1 Particles in Cell Culture Supernatants and Purified Virus Preparations

Art. No.: PRAAV1
Contents: 12 x 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, 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.

Immunotitrization by PROGEN’s AAV1 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV1 wt virions, AAV1 recombinant virions or assembled and intact empty AAV1 capsids.

The anti-AAV1 (ADK1) antibody cross-reacts with AAV6. The AAV1 Titration ELISA is, however, not validated for a titration of AAV6 capsids.

2. Test Principle

The assay is based on the sandwich ELISA technique. A monoclonal antibody (ADK1) specific for a conformational epitope on assembled AAV1 (and also AAV6) capsids is coated onto microtiter strips and is used to capture AAV1 and also AAV6 particles from the specimen. Captured AAV particles are detected in two steps. First a biotin-conjugated monoclonal antibody to AAV1 (ADK1) and also cross-reactive with AAV6 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 AAV1 particle preparation of 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 AAV1 sample preparation (e.g. infectious titer, DNA dot blot titer).

Table 1: Comparison of Titration Methods

<table>
<thead>
<tr>
<th>Titration Method</th>
<th>Titer</th>
<th>Corresponds to</th>
<th>Dilution in Titratio ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>infectious center assay</td>
<td>1×10⁸ IU/mL</td>
<td>1×10¹² P/mL</td>
<td>1:100 - 1:500</td>
</tr>
<tr>
<td>DNA dot blot assay</td>
<td>1×10¹⁰ drp/mL</td>
<td>1×10¹¹ P/mL</td>
<td>1:10 – 1:50</td>
</tr>
</tbody>
</table>

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 AAV1 in resealable aluminum bag with desiccant. Ready-to-use.
- KC  Kit Control (AAV1), lyophilized, 3 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 CON  Anti-AAV1 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 components except S and STOP contain a preservative!

a IU/mL: Infectious units/mL
b P/mL: Particles/mL
c drp/mL: DNAse resistant particles/mL
5. Preparation of Reagents

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 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-AAV1 biotin conjugate: Reconstitute B CON with 750 µL distilled water.

Immediately before use dilute 1:80 with ready-to-use wash buffer for ready-to-use AAV1 biotin conjugate.

Streptavidin peroxidase conjugate: Immediately before use dilute 1:20 with ready-to-use wash buffer for ready-to-use streptavidin peroxidase conjugate.

Reconstitution of kit control: Reconstitute with 500 µL distilled water; contains a defined amount of particles/mL (see label for exact concentration).

6. Stability of Reagents

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

Stability after opening:

6 months at 2-8°C:

WASH 20x, SB 20x, CON 20x, S

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

B CON, KC

7. Kit Control and Specimen Dilution

For the range of the ELISA see Table 2 and Fig. 1. Dilute specimen containing AAV1 particles to reach a concentration within the linear range of the ELISA using ready-to-use sample buffer.

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

Dilute the reconstituted kit control (KC) in ready-to-use sample buffer (see examples for dilution in Fig. 1 and Table 2).

### Table 2: Example for a serial dilution of Kit Control and the corresponding absorbance

<table>
<thead>
<tr>
<th>Kit Control</th>
<th>Capsids/mL</th>
<th>A 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted (1:1)</td>
<td>2.1 x 10^9</td>
<td>3.60</td>
</tr>
<tr>
<td>1:2</td>
<td>1.1 x 10^9</td>
<td>2.90</td>
</tr>
<tr>
<td>1:4</td>
<td>5.1 x 10^8</td>
<td>2.10</td>
</tr>
<tr>
<td>1:8</td>
<td>2.6 x 10^8</td>
<td>1.10</td>
</tr>
<tr>
<td>1:16</td>
<td>1.3 x 10^7</td>
<td>0.60</td>
</tr>
<tr>
<td>1:32</td>
<td>6.5 x 10^6</td>
<td>0.35</td>
</tr>
<tr>
<td>1:64</td>
<td>3.3 x 10^5</td>
<td>0.19</td>
</tr>
</tbody>
</table>

The values in this table correspond with Fig. 1.

### Fig. 1: Example of a Titration Curve

8. Test Procedure

1. Pipette 100 µL of ready-to-use sample buffer (Blank), serial dilutions of kit control 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 absorbent paper. Repeat washing step 2x.

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**

Calculate the average absorbance values for each set of duplicate kit control dilutions and duplicate samples.

Create a standard curve by plotting the mean absorbance value of each serial dilution of the kit control (y-axis) against the concentration of the corresponding diluted kit control (x-axis).

We suggest using a suitable computer program for the calculation of the particle titer of unknown specimens.

**10. Quality Control**

Kit Control (undiluted)   OD > 1.2
Blank             OD < 0.2

**11. Notes for the User**

**Security notes**

All components except S and STOP contain a 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. Such components or kits should be stored until the complaint is handled.

**11. Reference**