PROGEN BIOTECHNIK GMBH
Maaßstraße 30
69123 Heidelberg
Germany
Telefon +49 (0) 6221 / 8278 11
Telefax +49 (0) 6221 / 8278 24
Internet: www.progen.de
E-mail: info@progen.de

AAV 2 Titration ELISA

Enzyme Immunoassay for the Quantitative Determination of AAV Serotype 2 Particles in Cell Culture Supernatants and Purified Virus Preparations

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

For research use only

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 (1). AAV has been used in several clinical trials (e.g. FIX, CFTR, Parkinsons's, Canavan disease) showing no serious vector-related adverse effects (2). 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.

Immunotitration by PROGEN's AAV 2 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV 2 wt virions, AAV 2 recombinant virions or assembled and intact empty AAV 2 capsids (3).

Test Principle

The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV 2 capsids (4) is coated onto microtiter strips and is used to capture AAV 2 particles from the specimen. Captured AAV particles are detected in two steps. First a biotin conjugated monoclonal antibody to AAV 2 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 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 inhouse AAV 2 preparation (e.g. infectious titer, transducing units, DNA dot blot titer) (Table 1).

Table 1: Comparison of Titration methods

Ex. No.	Titration Method	Titer	Corresponds to	ELISA	
				Dilution	Expected OD Values
1	infectious center assay	1×10^{8} IU/mL*	10^{12} P/mL†	1:1,000	1.3
2	DNA dot blot assay	1×10^{10} drp/mL‡	1×10^{11} P/mL	1:100	1.3

Material Required

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

* Iu/mL: Infectious units/mL.
† P/ml: Particles/mL
‡ drp/mL: DNA resistant particles/mL.

Contents of Test Kit

MTP	Microtiter Plate, 12 × 8-well-strips, coated with mouse monoclonal antibody to AAV 2.
KC	Kit Control (empty AAV 2 capsids), lyophilised, 3 vials.
SB 20x	Sample Buffer, 20x, 20 mL.
WASH 20x	Wash Buffer 20x, 20 mL, 2 bottles.
B 20x	Anti-AAV 2 Biotin Conjugate 20x, lyophilised.
CON 20x	Streptavidin Peroxidase Conjugate 20x, liquid.
S (20x)	Substrate 20x, TMB (tetramethylbenzidine), 750 µl.
STOP	Stop Solution (ready-to-use), 13 mL
	Adhesion foil
	Resealable plastic bag.

All components except S (20x) and STOP contain 0.01%Thimerosal as preservative!

Preparation of Reagents

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

Store unused strips in a resealable plastic bag with desiccant at 2-8°C.

Dilute required volumes of reagents immediately before use!

Preparation of Reagents

***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 2 biotin conjugate[§]: Reconstitute with 750 µl distilled water.

Dilute 1:20 with ready-to-use wash buffer for ready-to-use AAV 2 biotin conjugate.

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

Substrate[§]: Dilute 1:20 with distilled water for ready-to-use substrate.

CAUTION: Dilute substrate in glass or polypropylene tubes only!

Reconstitution of Kit Control:

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

Kit Control and Specimen Dilution

The linear range of the ELISA covers 5×10^7 – 1×10^9 particles/mL. Dilute specimen containing AAV 2 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 **Kit Control** in ready-to-use sample buffer (see examples for dilution in Figure 1 and Table 2).

Fig. 1: Example of a Titration Curve of Kit Control

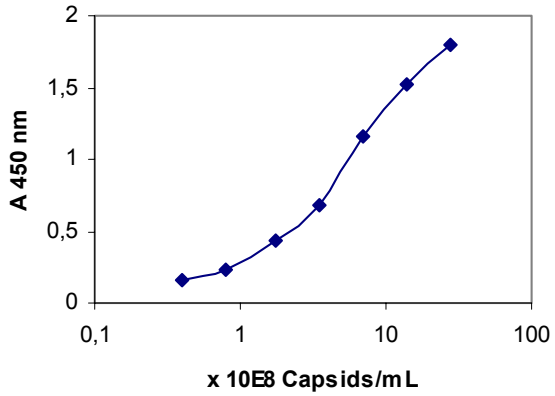

Table 2: Example of Titration of Kit Control

Kit Control	Capsids/mL	A 450 nm
Undiluted (1:1)	28 x 10^8	1.792
1:2	14 x 10^8	1.522
1:4	7 x 10^8	1.153
1:8	3.5 x 10^8	0.688
1:16	1.75 x 10^8	0.431
1:32	0.88 x 10^8	0.237
1:64	0.44 x 10^8	0.161

The values in this table correspond with Figure 1

§ Dilute immediately before use.

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 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 10 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.

Calculation of Results

Create a titration curve by using semilogarithmic paper and plotting the OD readings (y-axis) of the serial dilution of the Kit Control (x-axis) analogously to Fig. 1.

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

Quality Control

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

Notes for the User

Security notes

All components except S (20x) 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.

Literature

1. **Stilwell, J.L. and Samulski, R.J. 2003.** Adeno-Associated Virus Vectors for Therapeutic Gene Transfer. Biotechniques 34(1):148-150,152,154 passim.
2. **Flotte T.R. 2004.** Immune responses to Recombinant Adeno-Associated Virus Vectors : Putting Preclinical Findings into Perspective. Human Gene Therapy 15:716-717.
3. **Grimm, D., Kern, A., Pawlita, F., Ferrari, F., Samulski, R. and Kleinschmidt, J. 1999.** Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2. Gene Ther. 6:132-1330.
4. **Wobus, C.E., Hügle-Dörr, B., Girod, A., Petersen, G., Hallek, M and Kleinschmidt, J.A. 2000.** Monoclonal Antibodies against the Adeno-Associated Virus Type 2 (AAV-2) Capsid: Epitope Mapping and Identification of Capsid Domains Involved in AAV-2-Cell Interaction and Neutralization of AAV-2 Infection. J. of Virology Vol 74:19, 9281-9293.

Manufactured by:

PROGEN Biotechnik GmbH

Maaßstraße 30
69123 Heidelberg
Germany
Telephone +49 (0) 6221 / 8278 11
Telefax +49 (0) 6221 / 8278 24
www.progen.de
info@progen.de