



AAV8

Titration ELISA

Manual

Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of AAV serotype 8 particles in cell culture supernatants and purified virus preparations.

Catalog No.:	PRAAV8
Contents:	12 x 8 Determinations
Storage conditions:	2–8°C
Version:	12

For research use only.

PROGEN

Table of Contents

1. Introduction	2
2. Test Principle	3
3. Required Material	5
4. Test Kit Contents	6
5. Preparation of Reagents	7
6. Storage & Stability	9
7. Short Protocol	10
8. Kit Control and Specimen Dilution	12
9. Test Procedure	14
10. Calculation of Results	15
11. Test Validity	17
12. Test Characteristics	17
13. General Information	18
14. References	20

1. Introduction

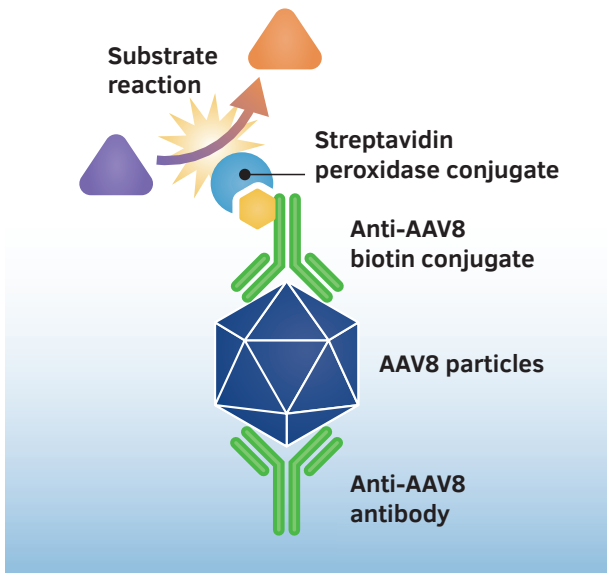
Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses, which are the subject of in-depth studies as viral vectors for gene therapy. The virus transduces a variety of dividing and non-dividing cells showing long-term gene expression with low cellular immune response. AAV has been used in several clinical trials (e.g. FIX, CFTR, Parkinson's, Canavan disease) showing no serious adverse vector-related effects.

Methods for characterizing AAV preparations currently include titration ELISA, qPCR, ddPCR, DNA dot blot, determination of transducing units, infectious center assay, SDS-PAGE and electron microscopy.

Immunotitration by PROGEN's AAV8 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV8 wild-type virions, AAV8 recombinant virions as well as assembled and intact empty AAV8 capsids.

2. Test Principle

The assay is based on the sandwich ELISA technique (see figure below). A monoclonal antibody specific for a conformational epitope on assembled AAV8 capsids (clone ADK8) is coated onto strips of a microtiter plate and is used to capture AAV8 particles from the specimen. Captured AAV particles are detected in two steps:



1. A biotin-conjugated monoclonal AAV8 (clone ADK8) antibody is bound to the immune complex.
2. A streptavidin peroxidase conjugate reacts with the biotin molecules.

Adding substrate solution results in a color reaction, which is proportional to the number of specifically bound viral particles. The absorbance is measured photometrically at 450 nm (optional: reference wavelength at 650 nm).

The provided Kit Control contains an AAV8 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. Required Material

Precision pipettes

Sterile pipette tips

Distilled water

Reaction tubes

Incubator at 37°C and, if necessary, incubator at room temperature (20-26°C)

ELISA Reader (450 nm, optional: reference wavelength at 650 nm)

4. Test Kit Contents

MTP	Microtiter Plate, 12 x 8-well-strips, coated with mouse monoclonal antibody to AAV8 in resealable aluminum bag with desiccant, 1 plate. Ready-to-use.
KC	Kit Control AAV8 (standard), lyophilized, 2 vials. Reconstitute before use.
ASSB 20x	Assay Buffer 20x, 3 x 20 ml. Dilute before use.
Biotin conc.	Anti-AAV8 Biotin Conjugate 20x, 1 vial, lyophilized. Reconstitute and dilute before use.
Strep-HRP 20x	Streptavidin Peroxidase Conjugate 20x, 750 µl. Dilute before use.
TMB	Substrate, TMB (tetramethylbenzidine), 12 ml. Ready-to-use.
STOP	Stop Solution, 13 ml. Ready-to-use.
Adhesive foil	

5. Preparation of Reagents

Prior to use, allow kit to reach room temperature (RT, 20–26°C).



Preparation and pre-dilution of components:

Dilute required reagent volumes immediately before use.

ASSB 20x (Assay Buffer 20x)

The buffer concentrate may contain salt crystals, which dissolve quickly at 37°C (e.g. in a water-bath). Let buffer cool down to RT before use.

1. Dilute **1:20** with distilled water.
2. The diluted component is named **ASSB 1x** (About 30 ml ASSB 1x per strip is needed).

KC (Kit Control)

1. Reconstitute with **750 µl ASSB 1x**.
2. Incubate for 5 min at RT and then mix by rolling for another 5 min. Avoid vortexing.
3. Find the amount of capsids/ml on the label or the lot-specific Quality Control Certificate.

Biotin conc. (Anti-AAV8 Biotin Conjugate 20x)

1. Reconstitute with **750 µl ASSB 1x**.
2. Incubate for 5 min at RT and then mix by rolling for another 5 min. Avoid vortexing.
3. Immediately before use, dilute **1:20** with **ASSB 1x**.
4. The diluted component is named **Biotin 1x**.

Strep-HRP 20x (Streptavidin Peroxidase Conjugate 20x)

1. Immediately before use, dilute **1:20** with **ASSB 1x**.
2. The diluted component is named **Strep-HRP 1x**.
3. Store in the dark until use.

6. Storage & Stability

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

Stability after opening:

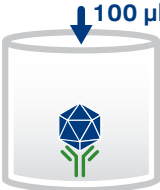
4 weeks at 2–8°C: ASSB 20x, Strep-HRP 20x, TMB, STOP

4 weeks after reconstitution at 2–8°C: KC, Biotin conc.

4 weeks in the resealable aluminum bag with desiccant at 2–8°C: MTP

7. Short Protocol

1 KC dilutions
Sample dilutions



100 µl

1 hour
37°C

The diagram shows a cylindrical well with a blue arrow pointing down into it labeled '100 µl'. Inside the well is a blue dodecahedron with green Y-shaped structures extending from its bottom. To the right of the well is a blue downward-pointing arrow containing the text '1 hour' and '37°C'.

 3 times 200 µl ASSB 1x

2 Biotin 1x




100 µl

1 hour
37°C

The diagram shows a cylindrical well with a blue arrow pointing down into it labeled '100 µl'. Inside the well is a blue dodecahedron with green Y-shaped structures extending from its bottom, and a yellow hexagon is positioned above the dodecahedron. To the right of the well is a blue downward-pointing arrow containing the text '1 hour' and '37°C'.

 3 times 200 µl ASSB 1x

3 Strep-HRP 1x



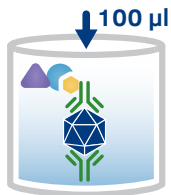
100 µl

1 hour
37°C

The diagram shows a cylindrical well with a blue arrow pointing down into it labeled '100 µl'. Inside the well is a blue dodecahedron with green Y-shaped structures extending from its bottom, a yellow hexagon is positioned above the dodecahedron, and a blue semi-circle is positioned to the left of the hexagon. To the right of the well is a blue downward-pointing arrow containing the text '1 hour' and '37°C'.

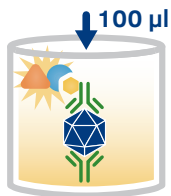
 3 times 200 µl ASSB 1x

4 TMB



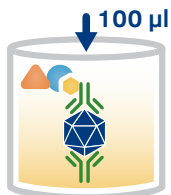
15 min
RT

5 Stop



RT

6 Read at 450 nm
(and 650 nm)



within
30 min

8. Kit Control and Specimen Dilution

We recommend diluting the reconstituted Kit Control (**KC**) in **ASSB 1x** in steps of 1:2:

Undiluted \rightarrow 1:2 \rightarrow 1:2 \rightarrow 1:4 \rightarrow 1:2 \rightarrow 1:8 \rightarrow 1:2 \rightarrow 1:16 \rightarrow 1:2 \rightarrow 1:32 \rightarrow 1:2 \rightarrow 1:64

An example for dilutions is provided in Table 1 on the lot-specific Example Curve document. Please find the lot-specific titer of the Kit Control on the vial or on the Quality Control Certificate. Both the Example Curve document and the Quality Control Certificate are provided with the kit.

Pre-dilute your **specimen** containing AAV8 particles in **ASSB 1x** in serial dilution steps to reach a concentration within the recommended quantification range of the ELISA (*please see section 10*). It might be necessary to perform a pre-experiment to determine the approximate titer of the unknown specimen before analyzing further dilutions.

See page 13 for an example of a plate layout.

Example for a plate layout:

	1	2	3	4	5	6	7	8
A	KC0	KC0	Sp1	Sp1	○	○	○	○
B	KC1	KC1	Sp2	Sp2	○	○	○	○
C	KC2	KC2	etc.	etc.	○	○	○	○
D	KC3	KC3	○	○	○	○	○	○
E	KC4	KC4	○	○	○	○	○	○
F	KC5	KC5	○	○	○	○	○	○
G	KC6	KC6	○	○	○	○	○	○
H	KC7	KC7	○	○	○	○	○	○

Sp1 = Specimen dilution 1 | Sp2 = Specimen dilution 2

Prepare dilutions:

KC0 ASSB 1x

KC1 reconstituted Kit Control

KC2 250 µl **KC1** + 250 µl ASSB 1x

KC3 250 µl **KC2** + 250 µl ASSB 1x

etc.

9. Test Procedure

1. Pipette 100 μ l of ASSB 1x (KC0), serial dilutions of KC and specimen (both in **ASSB 1x**) in duplicates into the corresponding wells of the microtiter strips. Seal strips with adhesive foil and incubate for **1 h at 37°C**.
2. Discard content of microtiter strips. For washing, fill each well with 200 μ l of **ASSB 1x**, incubate approximately 5 sec, discard and tap inverted plate onto absorbent paper. Carry out **three** washing steps in total.
3. Prepare Biotin 1x. Pipette 100 μ l of **Biotin 1x** into each well. Seal strips with adhesive foil and incubate for **1 h at 37°C**.
4. Repeat washing step as described in 2.
5. Prepare Strep-HRP 1x. Pipette 100 μ l of **Strep-HRP 1x** into each well. Seal strips with adhesive foil and incubate for **1 h at 37°C**.
6. Repeat washing step as described in 2.
7. Pipette 100 μ l of ready-to-use **TMB** into each well. Seal strips with adhesive foil and incubate for **15 min at RT**.

8. Stop color reaction by adding 100 μ l of **STOP** into each well.
9. Make sure no air bubbles are in the wells. **Within 30 min**, measure color intensity with a photometer at a wavelength of 450 nm (optional: reference wavelength at 650 nm).

10. Calculation of Results

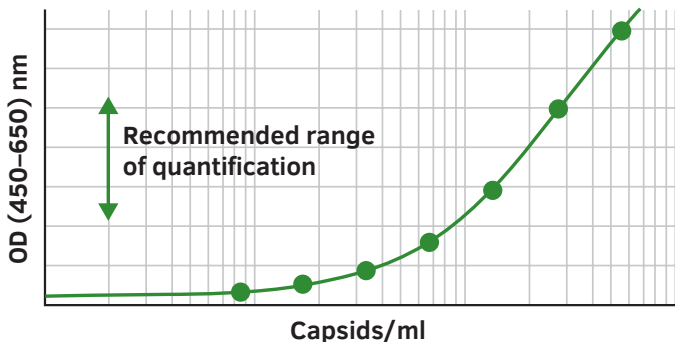
If applicable, subtract values measured at 650 nm reference wavelength from values at 450 nm. The test is also valid if you use OD values at 450 nm only.

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

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

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.

The kit is quantitative over the whole range of Kit Control dilutions. For highest accuracy, the OD values of unknown samples should ideally be in the recommended range for quantification:



Multiply the value obtained by the dilution factor to determine the amount of capsids/ml in the sample.



Please note:

The Kit Control curve needs to be determined for each experiment individually. For further guidance take a look at the lot-specific Example Curve provided with the kit.

11. Test Validity

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

The absorbance value of the Blank should be < 0.3 .

12. Test Characteristics

The Kit Control has been calibrated on the ATCC AAV8 RSM (VR-1816), [**Ayuso** *et al.*, 2014].

13. General Information

For professional use.

Release notes

The instruction manual is only valid in combination with the lot-specific documents (→ *Example Curve and Quality Control Certificate*), which are enclosed in each kit.

Please make sure to use the instruction manual with the version number that corresponds to the number on the lot-specific documents.

Precautions

All liquid components except TMB and STOP contain a preservative. Do not swallow. Avoid any contact with skin or mucous epithelia!

STOP (sulphuric acid) and TMB may cause skin or eye irritation. In the event of eye contact, rinse out immediately with plenty of water and consult a physician!

Safety data sheet is available on request.

Disposal

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 respective national regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified otherwise, non-contaminated packaging may be treated like household waste or may be recycled.

Transport damages

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

14. References

D'Costa, S. et al. Practical utilization of recombinant AAV vector reference standards: focus on vector genomes titration by free ITR qPCR. *Mol. Ther. Methods Clin. Dev.* 5, 16019 (2016).

Ayuso, E. et al. Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. *Hum. Gene Ther.* 25, 977–987 (2014).

Gurda, B. L. et al. Mapping a neutralizing epitope onto the capsid of adeno-associated virus serotype 8. *J. Virol.* 86, 7739–7751 (2012).

Sonntag, F. et al. The Assembly-Activating Protein Promotes Capsid Assembly of Different Adeno-Associated Virus Serotypes. *J. Virol.* 85, 12686–12697 (2011).



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