Version 11-04/24

User's Manual

AAV2 Titration ELISA Kit

REF **DEIA589**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of AAV serotype 2 particles in cell culture supernatants and purified virus preparations.

General Description

Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses, which are 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, qPCR, ddPCR, DNA dot blot, determination of transducing units, infectious center assay, SDS-PAGE or electron microscopy. Immunotitration by creative diagnostics AAV2 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV2 wild-type virions, AAV2 recombinant virions or assembled and intact empty AAV2 capsids.

Principles of Testing

The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV2 capsids is coated onto strips of a microtiter plate and is used to capture AAV2 particles from the specimen. Captured AAV particles are detected in two steps:

1. A biotin-conjugated monoclonal antibody to AAV2 is bound to the immune complex.
2. 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 (optional: reference wavelength at 620 nm). The provided Kit Control contains an AAV2 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.

Reagents And Materials Provided

1. Microtiter Plate, 12 × 8-well-strips, coated with mouse monoclonal antibody to AAV2 in aluminum bag with desiccant, 1 plate. Ready- to-use.
2. AAV2 standard, lyophilized, 3 vials. Reconstitute before use.
3. Washing Buffer 20×, 50 ml. Dilute before use.
4. Sample dilution, 50ml. Ready- to-use.
5. Anti-AAV2 Biotin Conjugate, lyophilized, 1 vial. Reconstitute before use.
6. Streptavidin Peroxidase Conjugate, 12ml. Ready- to-use.
7. TMB Substrate, 6 ml × 2. Ready-to-use.
8. Stop Solution, 7 ml. Ready-to-use.

Materials Required But Not Supplied

1. Precision pipettes
2. Sterile pipette tips
3. Distilled water
4. Reaction tubes
5. Incubator at at room temperature (20-25°C)
6. ELISA Reader (450 nm, optional: reference wavelength at 630 nm)

Storage

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

Specimen Collection And Preparation

We recommend to dilute the reconstituted AAV2 standard in Sample dilution in steps of 1:2:

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.

Prepare dilutions:
KC0: Sample dilution
KC1: reconstituted AAV2 Standard
KC2: 250µL KC1 + 250µL Sample dilution
KC3: 250µL KC2 + 250µL Sample dilution
etc.

Pre-dilute your specimen containing AAV2 particles in Sample dilution in serial dilution steps to reach a concentration within the recommended quantification range of the ELISA. It might be necessary to perform a pre-experiment to determine the approximate titer of the unknown specimen before analyzing more finetuned dilutions. Example for a plate layout:

	1	2	3	4
A	KC0	KC0	Specimen dilution 1	Specimen dilution 1
B	KC1	KC1	Specimen dilution 2	Specimen dilution 2
C	KC2	KC2	etc.	etc.
D	KC3	KC3		
E	KC4	KC4		
F	KC5	KC5		
G	KC6	KC6		
H	KC7	KC7		

Reagent Preparation

Prior to use, allow kit to reach room temperature (RT, 20- 25°C). Preparation and pre-dilution of components: Dilute required reagent volumes immediately before use!

Anti-AAV2 Biotin Conjugate

1. Reconstitute it with 1.2 mL ultrapure water as a 10× stock and vortex gently.
2. The reconstituted reagent should be aliquoted and stored below -20°C.
3. After addition of ultrapure water, diluent prepared 10× stock to 1× with Sample dilution (for example, add 1 mL 10× stock to 9 mL of Sample dilution for 1× in 10 mL). Mix well.

Washing Buffer 20×

1. Dilute 1:19 with distilled water.
2. The diluted component is named washing Buffer 1×

KC (Kit Control)

1. Reconstitute with 500 µl washing Buffer 1×.
2. Incubate for 5 min at RT and then mix by rolling for another 5 min. Avoid vertexing.
3. Find the amount of vg/ml on the label.

Assay Procedure

1. Pipette 100 µL of Sample dilution (KC0), serial dilutions of KC and specimen (both in Sample dilution) in duplicates into the corresponding wells of the microtiter strips. Seal strips with adhesive foil and incubate at 250rpm/RT for 2 h.
2. Discard content of microtiter strips. For washing, fill each well with 200 µL of washing buffer 1×, incubate

approximately 5 sec, discard and tap inverted plate onto absorbent paper. Carry out three washing steps in total.

3. Pipette 100 µL of Biotin into each well. Seal strips with adhesive foil and incubate at 250rpm/RT for 1 h.
4. Repeat washing step as described in 2.
5. Pipette 100 µL of Strep-HRP into each well. Seal strips with adhesive foil and incubate at 250rpm/RT for 1 h
6. Repeat washing step as described in 2.
7. Pipette 100 µL of ready-to-use TMB into each well. Seal strips with adhesive foil and incubate for 20 min at RT.
8. Stop color reaction by adding 50 µL 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 620 nm).

Calculation

1. If applicable, subtract values measured at 620 nm reference wavelength from values at 450 nm. The test is also valid if you use OD values at 450 nm only.
2. Calculate the average absorbance values for each duplicate set of Kit Control dilutions and specimen dilutions.
3. 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).
4. 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.
5. 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.
6. Multiply the value obtained by the dilution factor to determine the amount of vg/ml in the sample.

Please note: The standard curve needs to be determined for each experiment individually. For further orientation, please find the lot-specific Example Curve provided with the kit.

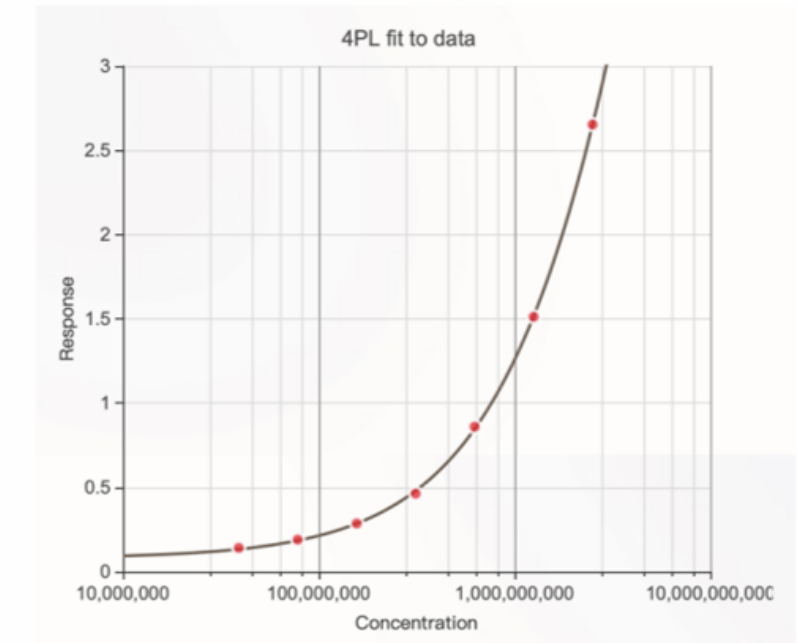
Test Validity

The absorbance value of the undiluted Kit Control should be > 1.5.

The absorbance value of the Blank should be < 0.15.

Typical Standard Curve

AAV2 Capsids vg/mL	OD450-620nm		
	(1)	(2)	Average
2.50E+09	2.671	2.635	2.653
1.25E+09	1.540	1.483	1.512
6.25E+08	0.844	0.875	0.860
3.13E+08	0.491	0.436	0.463
1.56E+08	0.294	0.278	0.286
7.81E+07	0.195	0.186	0.190
3.91E+07	0.140	0.141	0.141
0.00E+00	0.067	0.078	0.073



Precautions

For professional use.

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.

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!

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.

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.