Version 15-08/24

User's Manual

AAV5 Titration ELISA Kit

REF **DEIAAV5**

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 5 particles in cell culture supernatants and purified virus preparations.

Principles of Testing

The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled AAV5 capsids is coated onto strips of a microtiter plate and is used to capture AAV5 particles from the specimen. Captured AAV particles are detected in two steps:

1. A biotin-conjugated monoclonal antibody to AAV5 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 AAV5 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 AAV5 in aluminum bag with desiccant, 1 plate. Ready- to-use.
2. AAV5 standard, lyophilized, 3 vials. Reconstitute before use.
3. Assay Buffer 20×, 50 ml. Dilute before use.
4. Anti-AAV5 Biotin Conjugate, lyophilized, 1 vial. Reconstitute before use.
5. Antibody Diluent, 12ml. Ready-to-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 37°C
6. Microplate Mixer
7. ELISA Reader (450 nm, optional: reference wavelength at 620 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

Pre-dilute your specimen containing AAV5 particles in Assay Buffer1× 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.

Plate Preparation

Pipetting protocol									
	1	2	3	4	5	6	7	…	12
A	Std.7	Std.7	Specimen Dilution 1	Specimen Dilution 1					
B	Std.6	Std.6	Specimen Dilution 2	Specimen Dilution 2					
C	Std.5	Std.5	etc.	etc.					
D	Std.4	Std.4							
E	Std.3	Std.3							
F	Std.2	Std.2							
G	Std.1	Std.1							
H	Std.0	Std.0							

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!

Assay Buffer 20×

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

AAV5 Standard

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

We recommend to dilute the reconstituted AAV5 standard in Assay Buffer1× in steps of 1:2:

Prepare dilutions:
Std.0: Assay Buffer1×
Std.7: reconstituted AAV5 Standard
Std.6: 250µL Std.7 + 250µL Assay Buffer 1×
Std.5: 250µL Std.6 + 250µL Assay Buffer 1×
etc.

An example for dilutions is provided 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.

Anti-AAV5 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 Antibody Diluent (for example, add 1mL 10× stock to 9 mL of Antibody Diluent). Mix well.

Assay Procedure

1. Pipette 100 µl of Assay Buffer 1× (Std.0), serial dilutions of AAV5 standard and specimen (both in Assay Buffer 1×) in duplicates into the corresponding wells of the microtiter strips. Seal strips with adhesive foil and incubate for 1.5 h at 37°C, 500rpm.
2. Discard content of microtiter strips. For washing, fill each well with 250 µl of Assay Buffer 1×, incubate approximately 5 sec, discard and tap inverted plate onto absorbent paper. Carry out five washing steps in total.
3. Pipette 100 µl of Anti-AAV5 Biotin Conjugate into each well. Seal strips with adhesive foil and incubate for 1 h at 37°C, 500rpm.
4. Repeat washing step as described in 2.
5. Pipette 100 µl of Streptavidin Peroxidase Conjugate into each well. Seal strips with adhesive foil and incubate for 1 h at 37°C, 500rpm.
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 37°C.
8. Stop color reaction by adding 50 µl of Stop Solution into each well.
9. Make sure no air bubbles are in the wells. Within 5 min, measure color intensity with a photometer at a wavelength of 450 nm (optional: reference wavelength at 620nm.)

Quality Control

The absorbance value of the undiluted AAV5 Standard should be > 1.5.

The absorbance value of the Std.0 should be < 0.4.

Calculation

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.

Calculate the average absorbance values for each duplicate set of Standard AAV5 dilutions and specimen dilutions. Create a standard curve by plotting the mean absorbance value of each AAV5 Standard 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.

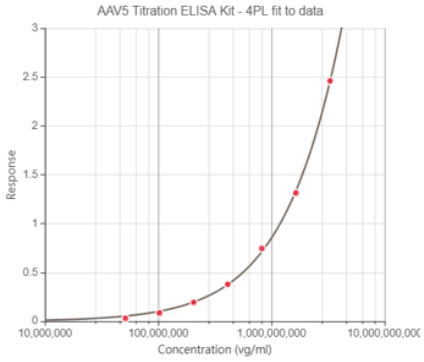
The kit is quantitative over the whole range of AAV5 Standard dilutions. For highest accuracy, the OD values of unknown samples should ideally be in the recommended range for quantification:

Multiply the value obtained by the dilution factor to determine the amount of 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.

Typical Standard Curve

AAV5 Capsids vg/mL	OD450-620nm			
	(1)	(2)	Average	Corrected
3.29E+09	2.5899	2.4541	2.5220	2.4608
1.65E+09	1.4357	1.313	1.3744	1.3132
8.23E+08	0.8169	0.7976	0.8073	0.7461
4.11E+08	0.444	0.4349	0.4395	0.3783
2.06E+08	0.2609	0.2484	0.2547	0.1935
1.03E+08	0.1461	0.1459	0.1460	0.0848
5.14E+07	0.0986	0.0822	0.0904	0.0292
0	0.06115	0.06115	0.0612	0.0000



$y = d + \frac{a-d}{1+(\frac{x}{c})^b}$	a	b	c	d	R^2
	0	0.939	4.13E+10	28.87	1

Detection Range

0-3.29E+09vg/mL

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.

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!

Chemicals and biological materials must be disposed of in compliance with the respective national regulations.