Product Manual

QuickTiter™ AAV Titer ELISA Kit

Catalog Number

VPK-5146	96 assays
VPK-5146-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Viral gene delivery systems include vectors developed from retrovirus (RV), adenovirus (AdV), adeno-associated virus (AAV), lentivirus (LV), and herpes simplex virus (HSV). AAV belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. There are eleven different AAV serotypes reported to date.

Recombinant AAV-2 is the most common serotype used in gene delivery, and it can be produced at high titers with a helper virus or Cell Biolabs' AAV Helper-Free System. AAV-2 can infect both dividing and non-dividing cells and can be maintained in the human host cell, creating the potential for long-term gene transfer. Because AAV-2 is a naturally defective virus, requiring provision of several factors in *trans* configuration for productive infection, it is considered the safest viral vector to use. Recently a new vector, AAV-DJ, was developed using DNA family shuffling to create a hybrid capsid from 8 different AAV serotypes, resulting in a vector with significantly higher *in vitro* infection rates across a variety of cells and tissues.

Recombinant AAV-2 and AAV-DJ vectors can be purified by CsCl gradient ultracentrifugation, iodixanol discontinuous gradient ultracentrifugation, or Cell Biolabs' ViraBind™ AAV Purification Kit.

A particular challenge in the delivery of a gene by a viral vector is the accurate measurement of virus titer. Traditionally, AAV particles are quantitated in number of genome copies (GC) by DNA dot blot or similar approaches. These methods are time-consuming and suffer from a high degree of inter-assay variability. For highly purified virus samples, an optical absorbance at 260 nm has been used to estimate the total number of virus particles. However, this method cannot be used in an unpurified viral supernatant, because other components in the crude supernatant can contribute to the optical absorbance of 260 nm.

Cell Biolabs QuickTiter™ AAV Titer ELISA Kit provides a quick and complete system to measure the titer of purified AAV viral samples; it provides sufficient reagents for up to 96 tests in a 96-well plate. Detection sensitivity is 6.25×10^8 viral particles (VP/mL) which is sufficient for most purified AAV samples. The provided AAV Standard contains a purified and chemically inactivated preparation of AAV particles. Performance of the standard curve allows for the quantitative determination of samples of an unknown purified AAV particle titer.

Assay Principle

The AAV capsid structure is made up of a mixture of three proteins named VP1, VP2, and VP3. There are a total of 60 monomer VP molecules per viral particle in a ratio of 5 VP1: 5 VP2: 50 VP3 (see ref. 4). An anti-AAV VP capsid protein monoclonal coating antibody capable of binding all three monomer subunits is adsorbed onto a microtiter plate. AAV capsid monomers present in the inactivated sample or standard bind to the antibodies adsorbed on the plate; an anti-AAV VP capsid polyclonal antibody capable of binding all three monomers is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, an HRP-conjugated secondary antibody is added that binds to the anti-AAV VP capsid polyclonal antibody. Unbound HRP-conjugated secondary antibody is removed during the wash steps, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of viral particles present in the sample.

The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from inactivated AAV standard and sample concentration is then determined.

It recognizes the following serotypes: AAV-1, AAV-2, AAV-3, AAV-5, AAV-6, AAV-8, AAV-9, AAV-10, AAV-DJ and AAV-DJ/8. The kit may be used with any recombinant AAV expression system as long as the virus sample is purified first.

Related Products

1. AAV-100: 293AAV Cell Line
2. AAV-200: ViraDuctin™ AAV Transduction Reagent
3. AAV-201: ViraDuctin™ AAV Transduction Reagent
4. VPK-140: ViraBind™ AAV Purification Kit
5. VPK-141: ViraBind™ AAV Purification Mega Kit
6. VPK-145: QuickTiter™ AAV Quantification Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-AAV Antibody Coated Plate (Part No. 51461B): One 96-well strip plate (8 x 12).
2. Anti-AAV Antibody (1000X) (Part No. 51462C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 231009): One 20 µL vial
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Lysis Buffer (Part No. 51463A): One 10 mL bottle containing 10% SDS.
6. 25X Salt Buffer (Part No. 51464A): One 5 mL bottle containing 0.5 M Tris pH 7.5 and 2.5 M NaCl.
7. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
9. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Inactivated AAV Standard (Part No. 51465D): One 100 µL vial of chemically and heat inactivated AAV at 1 x 10^{12} VP/mL.

Materials Not Supplied

1. Recombinant Purified AAV Samples
2. PBS

3. Microcentrifuge
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store the 10X Lysis Buffer at room temperature. Store the Anti-AAV Antibody (1000X) at -20°C. Store the Inactivated AAV Standard at -80°C. Store all other kit components at 4ºC.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-AAV Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-AAV Antibody and the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Inactivated AAV standards in the concentration range of 0 to 4 x10^{10} VP/mL into Assay Diluent (Table 1).

Standard Tubes	1 x 10^{12} VP/mL Inactivated AAV Standard (µL)	Assay Diluent (µL)	AAV (x10^{10} VP/mL)
1	20	480	4
2	250 of Tube #1	250	2
3	250 of Tube #2	250	1
4	250 of Tube #3	250	0.5
5	250 of Tube #4	250	0.25
6	250 of Tube #5	250	0.125
7	250 of Tube #6	250	0.063
8	0	250	0

Table 1. Preparation of Inactivated AAV Standards

Lysis and Heat Inactivation of Purified AAV Samples

1. (optional) For unknown purified viral samples, properly dilute viral sample with PBS.
2. Add 225 µL of each purified AAV unknown sample to a microcentrifuge tube.

3. Add 25 µL of 10X Lysis Buffer to each purified AAV unknown sample and mix well.
4. Incubate each mixed sample at 100°C for 5 minutes.
5. Transfer each sample to room temperature for 5 minutes.
6. Briefly centrifuge each sample for 30 seconds. Store unused prepared sample at room temperature.

Assay Protocol

1. Add 100 µL of the above lysed and heat inactivated AAV unknown sample, standard, or blank to the Anti-AAV Antibody Coated Plate. Each AAV unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted anti-AAV antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the QuickTiter™ AAV Titer ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

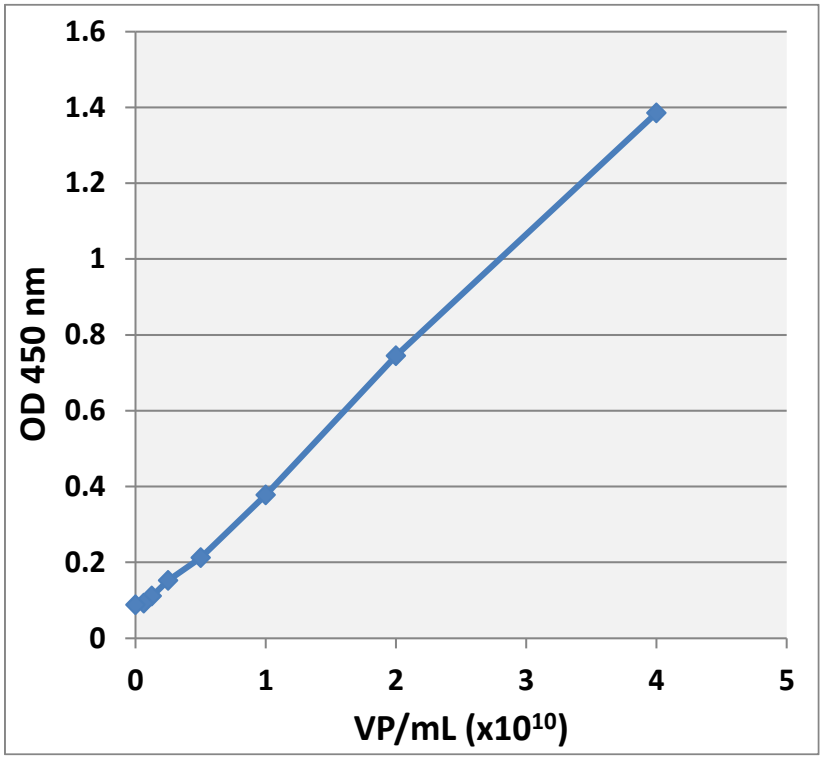

Figure 1: QuickTiter™ AAV Titer ELISA Kit Standard Curve.

References

1. Rabinowitz, J, and Samulski, R. J. (1998) *Curr. Opin. Biotechnol*., **9**, 470-475.
2. Summerford, C., and Samulski, R. J. (1999) *Nat. Med., **5**, 587-588.*
3. Clark, K., Liu, X., McGrath, J., and Johnson, P. (1999) *Hum. Gene Ther*., **10**, 1031-1039.
4. Sonntag, F., Schmidt, K., and Kleinschmidt, J.A. (2010) *Proc Natl Acad Sci U S A.*, **107**, 10220-5.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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