Product Manual

QuickTiter[™] AAV Titer ELISA Kit

Catalog Number

VPK-5211 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' ViraBindTM 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. The kit has a detection sensitivity of 3.1 x 10⁸ viral particles/mL (VP/mL), which is sufficient for most purified AAV samples. Each kit provides sufficient reagents to perform up to 96 assays including AAV standard curve and unknown purified AAV samples.

Assay Principle

The AAV capsid structure is made up of a mixture of three viral proteins (VP), 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 antibody capable of binding all three VP subunits is absorbed onto a microtiter plate. AAV VP present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-AAV VP polyclonal antibody is added and binds to the AAV VP captured by the first antibody. Following incubation and wash steps, a Streptavidin-Enzyme conjugate is added that binds to the anti-AAV VP polyclonal antibody. Unbound Streptavidin-Enzyme conjugate is removed during the wash steps, and substrate solution is added to the wells. A colored product is formed in proportion to the number of AAV viral particles present in the sample. The reaction is terminated by addition of Stop Solution and absorbance is measured at 450 nm. A standard curve is prepared from inactivated AAV standard and sample concentration is then determined.

QuickTiter[™] AAV Titer ELISA Kit 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.



Related Products

- 1. VPK-5202: AAV VP ELISA Kit
- 2. AAV-100: 293AAV Cell Line
- 3. AAV-200: ViraDuctin[™] AAV Transduction Reagent
- 4. VPK-140: ViraBindTM AAV Purification Kit
- 5. VPK-145: QuickTiter[™] AAV Quantification Kit

Kit Components

Box 1 (shipped at room temperature)

- 1. <u>Anti-AAV VP Antibody Coated Plate</u> (Part No. 52111B): One 96-well strip plate (8 x 12).
- 2. <u>Biotinylated Anti-AAV VP Antibody (1000X)</u> (Part No. 52112C): One 10 µL vial.
- 3. <u>Streptavidin-Enzyme Conjugate</u> (Part No. 310803): One 20 µL vial.
- 4. Assay Diluent (Part No. 310804): One 50 mL bottle.
- 5. <u>10X AAV Lysis Buffer</u> (Part No. 52113A): One 10 mL bottle containing 10% SDS.
- 6. <u>10X Wash Buffer</u> (Part No. 310806): One 100 mL bottle.
- 7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
- 8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. <u>Inactivated AAV2 Standard</u> (Part No. 52114D): One 100 μ L vial of chemically and heat inactivated AAV2 at 5 x 10¹¹ VP/mL.

Materials Not Supplied

- 1. Recombinant Purified AAV Samples
- 2. PBS
- 3. Microcentrifuge
- 4. Microplate reader capable of reading at 450 nm

Storage

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

Safety Considerations

Remember that you will be working with unknown 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.
- Biotinylated anti-AAV VP Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the biotinylated anti-AAV VP Antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of inactivated AAV2 standards in the concentration range of 0 to 2×10^{10} VP/mL into Assay Diluent (Table 1).

Standard Tubes	5 x 10 ¹¹ VP/mL Inactivated AAV2 Standard (μL)	Assay Diluent (µL)	AAV2 (x10 ¹⁰ VP/mL)
1	20	480	2
2	250 of Tube #1	250	1
3	250 of Tube #2	250	0.5
4	250 of Tube #3	250	0.25
5	250 of Tube #4	250	0.125
6	250 of Tube #5	250	0.063
7	250 of Tube #6	250	0.031
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 μ L of each purified AAV unknown sample to a microcentrifuge tube.
- 3. Add 25 μ L 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.
- Dilute sample 1:10 with Assay Diluent or PBS containing 0.1% BSA, assay immediately or store diluted sample at -80°C

Note: SDS/heat inactivated samples must be diluted 1:10 to lower the SDS concentration before used in the ELISA below.



Assay Protocol

- Add 100 μL of the above lysed and heat inactivated AAV unknown sample, standard, or blank to the Anti-AAV VP 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.
- 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 biotinylated anti-AAV VP 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 Streptavidin-Enzyme 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.
- 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.

- 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: QuickTiterTM AAV Titer ELISA Kit Standard Curve using the inactivated AAV2 Standard.



Figure 2: QuickTiter[™] AAV Titer ELISA Kit detection of inactivated AAV-DJ virus.



A: AAV VP Isoforms



B: AAV2 VP1 Sequence

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPS QMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT PSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEY SWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKT NVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKN TPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY TSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

Figure 3. AAV VP isoforms and antigen specificity. C-terminal conserved sequence (Asp469-Leu735, highlighted in red at 3A and underlined at 3B) of VP1, VP2 and VP3 was used as an immunogen to make the anti-AAV VP Antibody.





Figure 4: Purification of Recombinant anti-AAV VP Antigen (Asp469-Leu735). Lane 1: SeeBlue Plus2 MW standard (Invitrogen); Lane 2: Ni-NTA Elution Fraction for Recombinant AAV2 VP Antigen. Recombinant AAV2 VP Antigen was used as an immunogen to produce the ELISA antibodies.

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 USA., 107, 10220-5.

<u>Warranty</u>

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