Product Manual

AAV Rep ELISA Kit

Catalog Number

VPK-5118 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Adeno-associated virus (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. The AAV genome is built of single-stranded deoxyribonucleic acid (ssDNA), either positive- or negative-sensed, which is about 4.7 kilobase long. The genome is made up of inverted terminal repeats (ITRs) at both ends of the DNA strand as well as two open reading frames (ORFs): Cap (Capsid) and Rep (Replication). The Cap coding region contains overlapping nucleotide sequences of the capsid proteins VP1, VP2 and VP3, which interact together to form an icosahedral capsid structure.

On the 5' end of the genome there are two promoters called p5 and p19, from which two overlapping mRNAs of different length can be produced. Each of the mRNA sequences contains an intron that can be either spliced out or not resulting in four different mRNAs, and therefore four possible Rep proteins. The protein names describe their size in kilodaltons (kDa): Rep78, Rep68, Rep52 and Rep40 (Figure 1). Rep78 and Rep68 bind the hairpin structure of the ITR and cleave the terminal resolution site within the hairpin. These two proteins were also shown to be required for integration of the AAV genome. All four Rep proteins have been shown to contain helicase activity and can bind ATP. These proteins have also been shown to increase transcription from the p40 promoter and decrease transcription from both the p5 and p19 promoters.

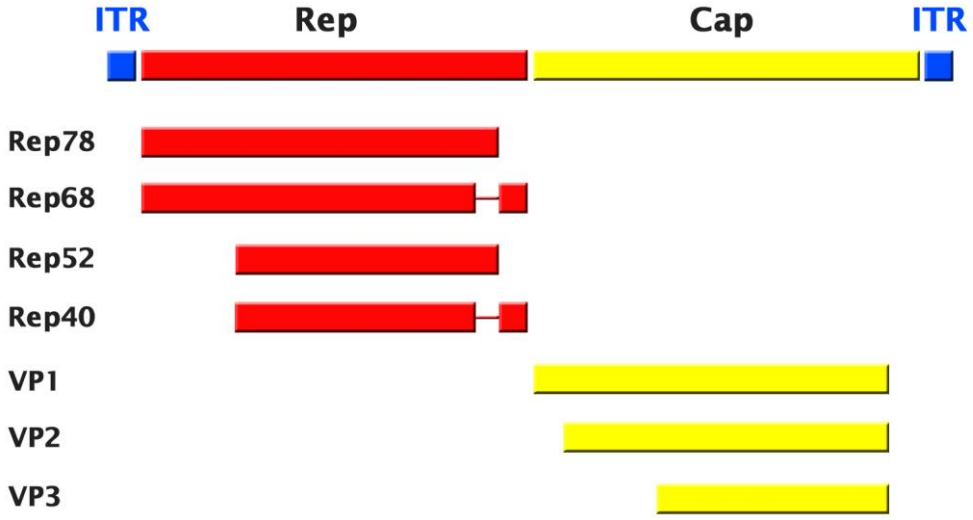

Figure 1. Schematic Map of AAV Genome. Rep: involved in genome replication.

Cell Biolabs' AAV Rep ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of all 4 rep proteins regardless of AAV serotype. The kit has a detection sensitivity limit of 30 pg/mL Rep protein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. AAV-100: 293AAV Cell Line
2. AAV-200: ViraDuctin™ AAV Transduction Kit

3. VPK-140: ViraBind™ AAV Purification Kit
4. VPK-141: ViraBind™ AAV Purification Mega Kit
5. VPK-145: QuickTiter™ AAV Quantitation Kit
6. VPK-410-SER2: AAV-2 Helper Free Expression System
7. VPK-400-DJ: AAV-DJ Helper Free Packaging System
8. VPK-400-DJ-8: AAV-DJ/8 Helper Free Packaging System

Kit Components

Box 1 (shipped at room temperature)

1. Anti-AAV Rep Antibody Coated Plate (Part No. 51181B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-AAV Rep Antibody (1000X) (Part No. 51182C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. AAV Rep Standard (Part No. 51183D): One 50 µL vial of 1 µg/mL recombinant AAV Rep (AAV2 Rep78 amino acids 383-529) in TBST plus BSA.

Materials Not Supplied

1. AAV Rep Samples: Cell or Tissue Lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at a wavelength of 450 nm

Storage

Upon receipt, aliquot and store the AAV Rep Standard at -80ºC and avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-AAV Rep Antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

- Biotinylated Anti-AAV Rep Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-AAV-Rep 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 AAV Rep standards in the concentration range of 0 to 2 ng/mL into Assay Diluent (Table 1).

Standard Tubes	1 µg/mL AAV Rep Standard (µL)	Assay Diluent (µL)	AAV Rep (pg/mL)	AAV Rep (pM)
1	2	998	2000	112
2	500 of Tube #1	500	1000	56
3	500 of Tube #2	500	500	28
4	500 of Tube #3	500	250	14
5	500 of Tube #4	500	125	7
6	500 of Tube #5	500	62.5	3.5
7	500 of Tube #6	500	31.25	1.75
8	0	500	0	0

Table 1. Preparation of AAV Rep Standards

Assay Protocol

1. Add 100 µL of AAV Rep unknown sample, standard, or blank to the Anti-AAV Rep Antibody Coated Plate. Each AAV Rep 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 Biotinylated Anti-AAV Rep 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.

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 AAV Rep ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

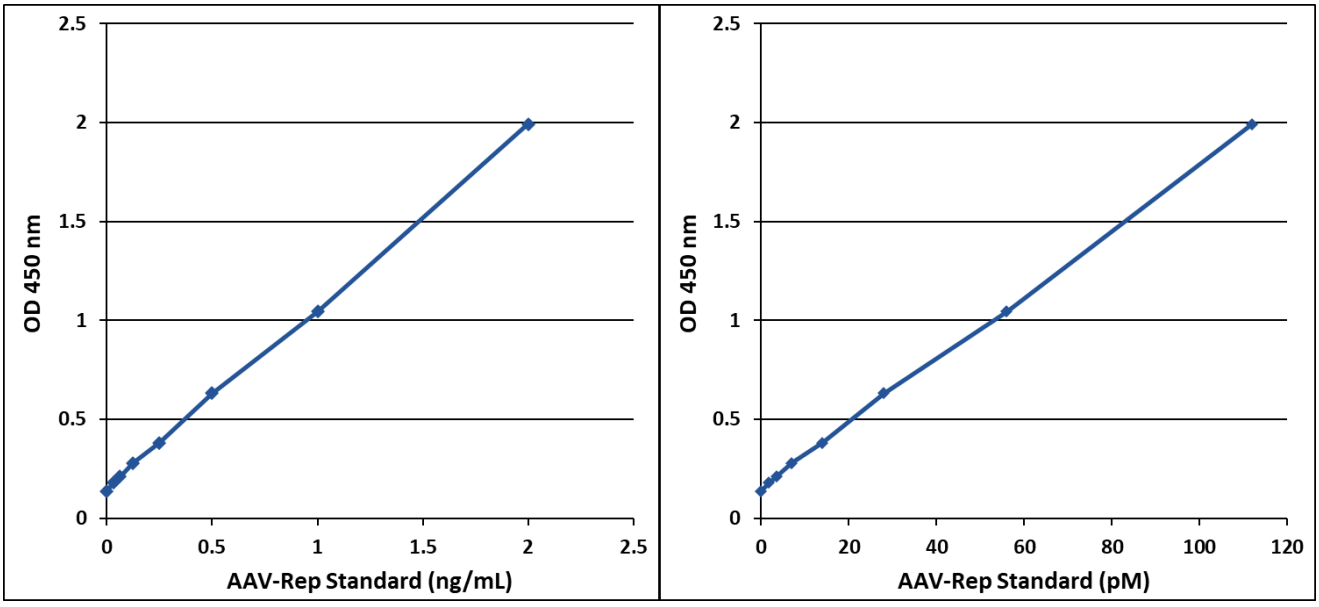

Figure 2: AAV Rep ELISA Kit Standard Curve.

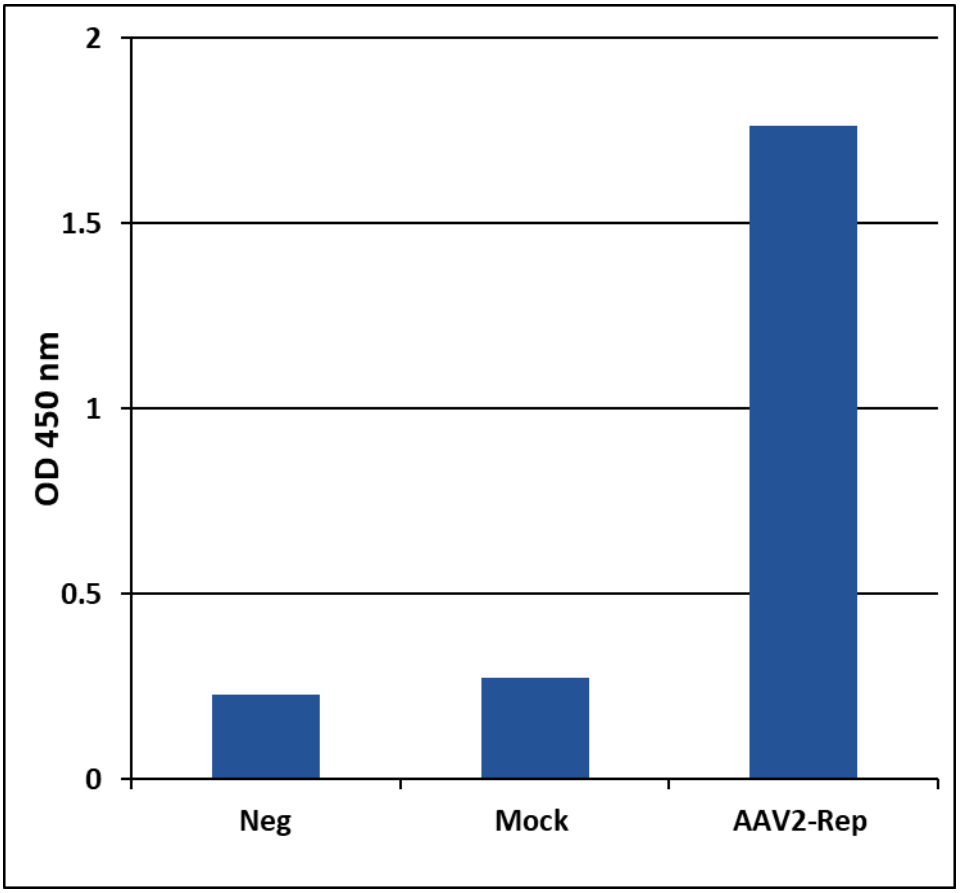

Figure 3: Detection of Rep Proteins in Transfected 293 cells. Cells were transiently transfected with pAAV-RC2 (catalog number VPK-422) or mock transfected. After 48 hours, cells were lysed in RIPA buffer and protein concentration was determined. The AAV Rep ELISA kit was performed in the absence of cell lysate (Neg), 600 ng lysate from mock transfected cells (Mock), or from AAV2-pAAV-RC2 transfected cells (AAV2-Rep).

A. Rep Protein Alignment

B. Recombinant AAV Rep Sequence

MTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQ
PLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWAKDHVVEVEHEFYVKKGGAKKRPAP
SDADISEPKRVRESVAQPSTSDAEASINYADRAAALEHHHHHH

Figure 4: Alignment of AAV Rep proteins. Conserved sequence used to make the recombinant AAV Rep Standard, also antibody immunogen, is highlighted in red (4A). Recombinant AAV Rep protein Sequence is underlined (4B).

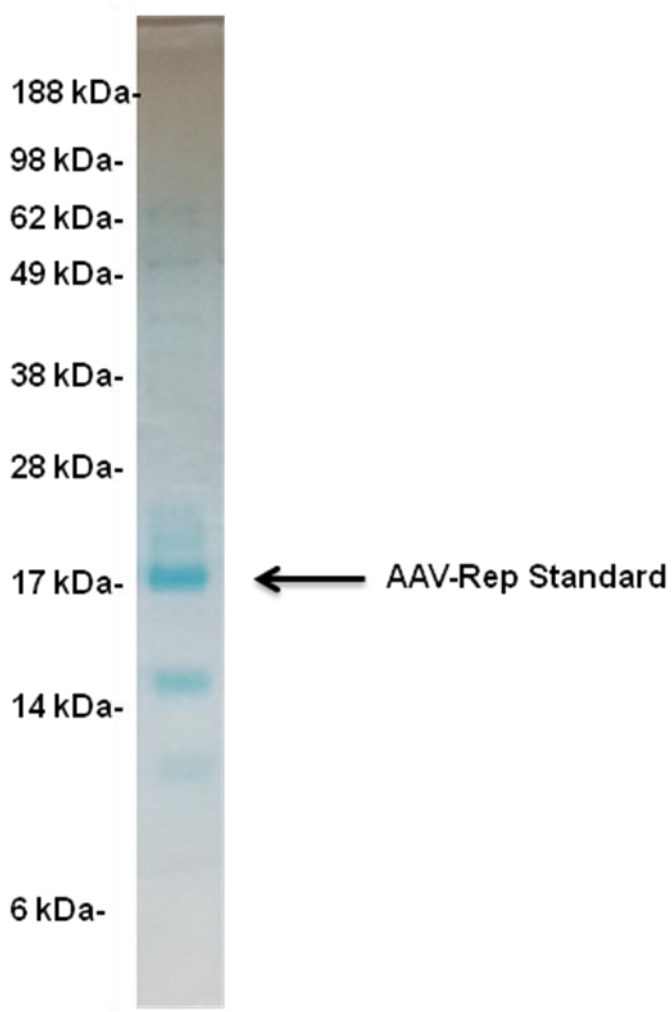

Figure 5: Purification of Recombinant AAV Rep Protein. Lane 1: SeeBlue Plus2 MW standard (Invitrogen); Lane 2: Ni-NTA Elution Fraction for Recombinant AAV Rep Protein. Purified recombinant AAV Rep protein was used as an immunogen to produce the ELISA antibodies.

References

1. Carter BJ (2000). *Gene Therapy: Therapeutic Mechanisms and Strategies*. 41–59.
2. Weitzman MD, Kyöstiö SR, Kotin RM, and Owens RA (1994). *PNAS USA*. **91**: 5808–12
3. Kyöstiö SR, Owens RA, Weitzman MD, Antoni BA, Chejanovsky N, and Carter BJ (1994). *J. Virol*. **68**: 2947–57
4. Im DS and Muzyczka N (1990). *Cell*. **61**: 447–57.
5. Im DS and Muzyczka N (1992). *J. Virol*. **66**: 1119–28
6. Trempe JP and Carter BJ (1988). *J. Virol*. **62**: 68–74

Warranty

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