Product Manual

AAV VP ELISA Kit

Catalog Number

VPK-5202 **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). 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. The Cap coding region contains overlapping nucleotide sequences of three capsid viral proteins (VP), VP1, VP2 and VP3, which interact together to form an icosahedral capsid structure in a ratio of 5 VP1: 5 VP2: 50 VP3 (Figure 1).

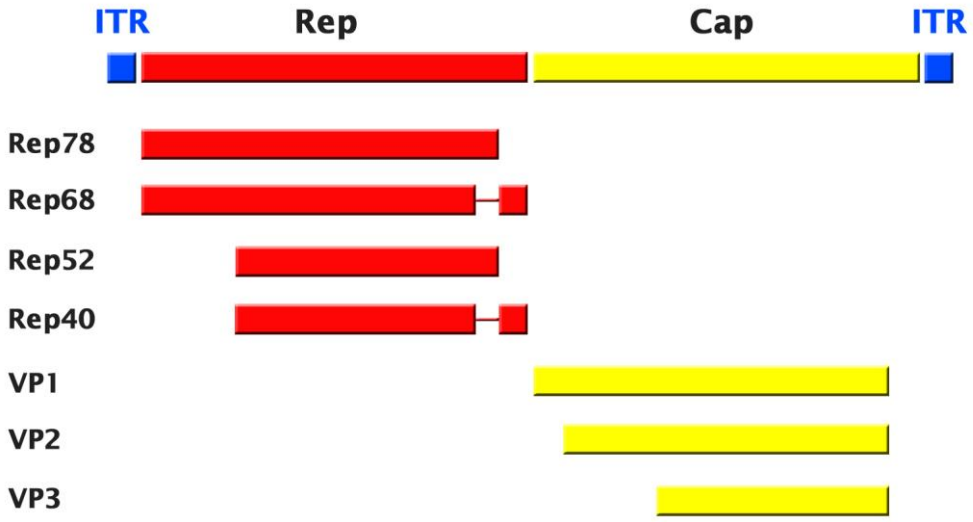


Figure 1. Schematic Map of AAV Genome. Rep: involved in genome replication; VP1/2/3: capsid viral proteins.

Cell Biolabs' AAV VP ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of capsid viral proteins (VP) regardless of AAV serotype. The kit has a detection sensitivity limit of 3.9 ng/mL VP. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. VPK-5118: AAV Rep ELISA Kit
2. VPK-5146: QuickTiter™ AAV Titer ELISA kit
3. AAV-200: ViraDuctin™ AAV Transduction Reagent
4. VPK-140: ViraBind™ AAV Purification Kit

5. VPK-145: QuickTiter™ AAV Quantification Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-AAV VP Antibody Coated Plate (Part No. 52111B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-AAV VP Antibody (1000X) (Part No. 52112C): 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 AAV Lysis Buffer (Part No. 52113A): One 10 mL bottle containing 10% SDS.
6. 10X Wash Buffer (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. AAV VP Standard (Part No. 52021B): One 100 µL of recombinant C-terminal AAV2 VP (Asp469-Leu735) at 12.5 µg/mL

Materials Not Supplied

1. Recombinant 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 all other kit components at 4ºC. Avoid multiple freeze/thaw cycles.

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.
- 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 VP standards in the concentration range of 0 to 250 ng/mL into Assay Diluent (Table 1).

Standard Tubes	12.5 µg/mL AAV VP Standard (µL)	Assay Diluent (µL)	VP Standard (ng/mL)
1	10	490	250
2	250 of Tube #1	250	125
3	250 of Tube #2	250	62.5
4	250 of Tube #3	250	31.3
5	250 of Tube #4	250	15.6
6	250 of Tube #5	250	7.8
7	250 of Tube #6	250	3.9
8	0	250	0

Table 1. Preparation of VP 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.
7. 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

1. Add 100 µL of the AAV VP unknown sample, standard, or blank to the Anti-AAV VP Antibody Coated Plate. Each AAV VP 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 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.
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 VP ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

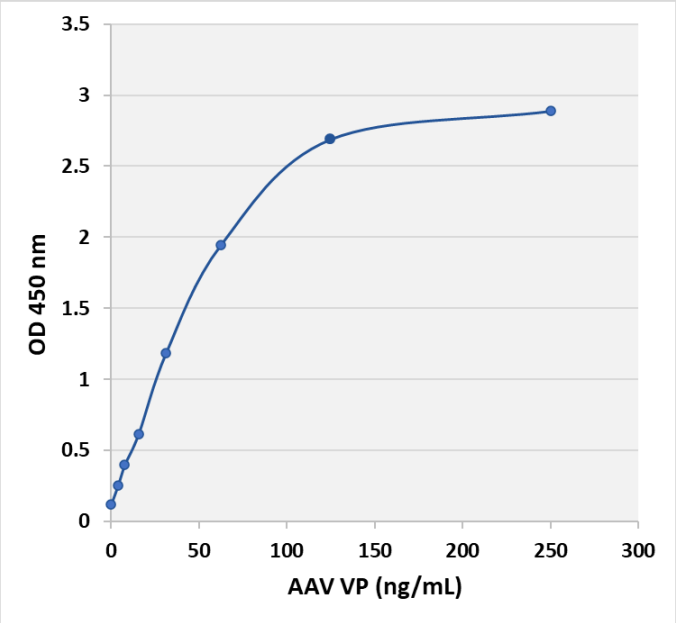
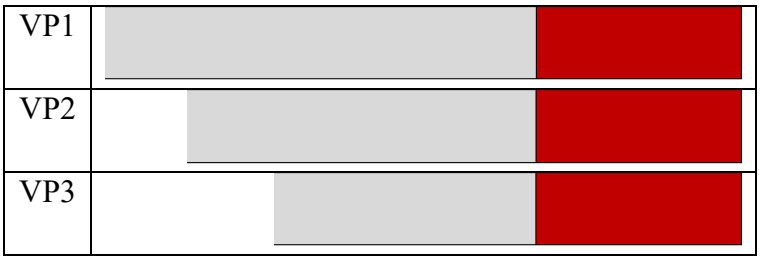


Figure 1: AAV VP ELISA Kit Standard Curve.

A: AAV VP Isoforms



B: AAV2 VP1 Sequence

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP
TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL
PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPS
QMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT
PSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEY

SWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKT
NVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV
LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKN
TPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY
TSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

Figure 2. 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.

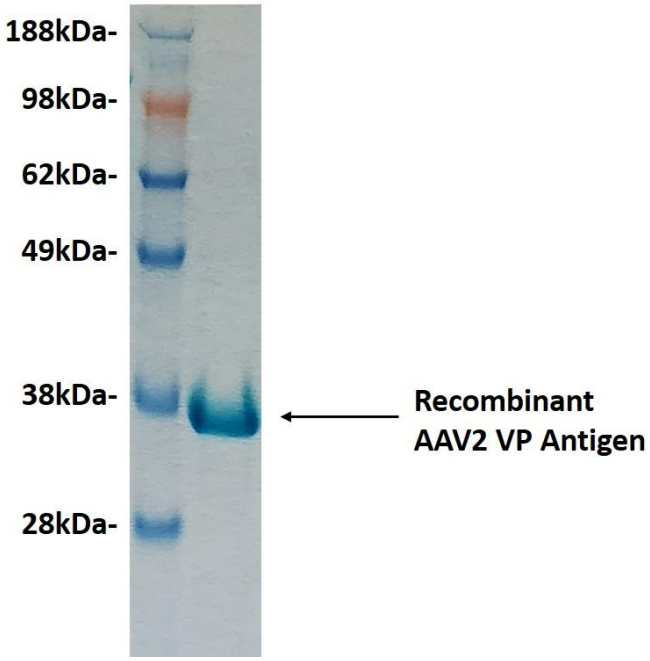


Figure 3: Purification of Recombinant anti-AAV VP Antigen (Asp469-Leu735). Lane 1: SeeBlue Plus2 MW standard (Invitrogen); Lane 2: Elution Fraction for Recombinant AAV2 VP Antigen. Recombinant AAV2 VP Antigen was used as an immunogen to produce the ELISA antibodies.

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 U S A.*, **107**, 10220-5.

Warranty

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