Product Manual

Adenovirus Hexon ELISA Kit

Catalog Numbers

VPK-5169 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Adenoviruses are non-enveloped viruses with icosahedral capsids of 90 to 100 nm in size containing a double stranded DNA genome ranging from 26 to 46 kb. Recombinant adenoviruses have been developed as gene delivery vectors for recombinant vaccines and gene therapy applications, including the treatment of metabolic disorders and cancers.

Hexon is the largest and most abundant of the structural protein in the icosahedral adenovirus capsid. Each adenoviral particle contains 240 copies of the hexon trimer. The other two major capsid proteins, the penton base and fiber, form the penton complex at each virion vertex.

Cell Biolabs' Adenovirus Hexon ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the adenovirus hexon protein. The ELISA antibodies against adenoviral hexon protein recognizes all 51 serotypes of adenovirus. The kit has a detection sensitivity limit of 156 pg /mL hexon. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and hexon samples.

Assay Principle

An anti-adenoviral hexon monoclonal coating antibody is adsorbed onto a microtiter plate. Adenoviral hexon present in the sample or standard binds to the antibodies adsorbed on the plate; an FITC-conjugated anti-hexon polyclonal antibody is added and binds to the hexon antigen captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti-hexon polyclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of adenovirus hexon 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 hexon standard and sample hexon concentration is then determined.

Related Products

1. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
2. VPK-150: QuickTiter™ Hepatitis B Core Antigen (HBcAg) ELISA Kit
3. VPK-151: QuickTiter™ Hepatitis C Core Antigen (HCcAg) ELISA Kit
4. VPK-156: QuickTiter™ MuLV Core Antigen (MuLV p30) ELISA Kit
5. VPK-5170: RSV Fusion Protein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Hexon Antibody Coated Plate (Part No. 51691B): One strip well 96-well plate.
2. FITC-Conjugated Anti-Hexon Polyclonal Antibody (Part No. 51692C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.

4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Viral Lysis Buffer (Part No. 51693B): One 15 mL bottle containing 200 mM Tris, pH 7.5, 1500 mM NaCl, 10% Triton X-100, 1% 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. Adenovirus Hexon Standard (Part No. 51694D): One 100 µL vial of 1 µg/mL Ad5 Hexon in PBS containing BSA.

Materials Not Supplied

1. Adenoviral Sample: purified virus or unpurified viral supernatant
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store the Adenovirus Hexon Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your adenoviral samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-Hexon Polyclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of Hexon Standard in the concentration range of 10 ng/mL – 0.156 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Hexon Standard (µL)	Assay Diluent (µL)	Hexon (ng/mL)
1	10	990	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 1. Preparation of Hexon Standard

2. Transfer 225 µL of each dilution to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer. Perform the assay as described in Assay Protocol.

Adenovirus Sample Inactivation and Lysis

1. (Optional) Dilute Adenovirus samples in culture medium. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer, vortex well. Inactivate Adenovirus sample at 56ºC for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as adenoviral lysate.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each adenovirus lysate sample, hexon standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of adenoviral lysate or hexon standard to Anti-Hexon Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 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.
6. Add 100 µL of the diluted FITC-Conjugated Anti-Hexon Polyclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.

9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. 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.
13. 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).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Adenovirus Hexon ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

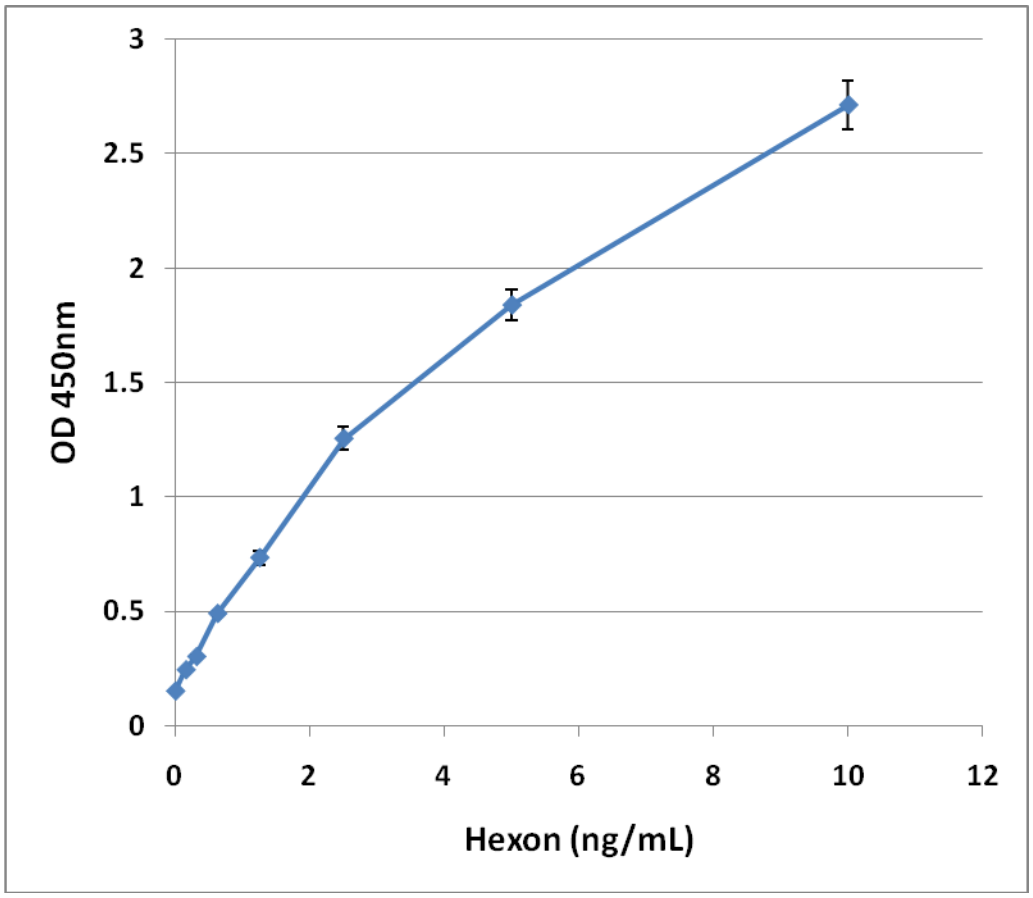

Figure 1: Adenovirus Hexon ELISA Standard Curve

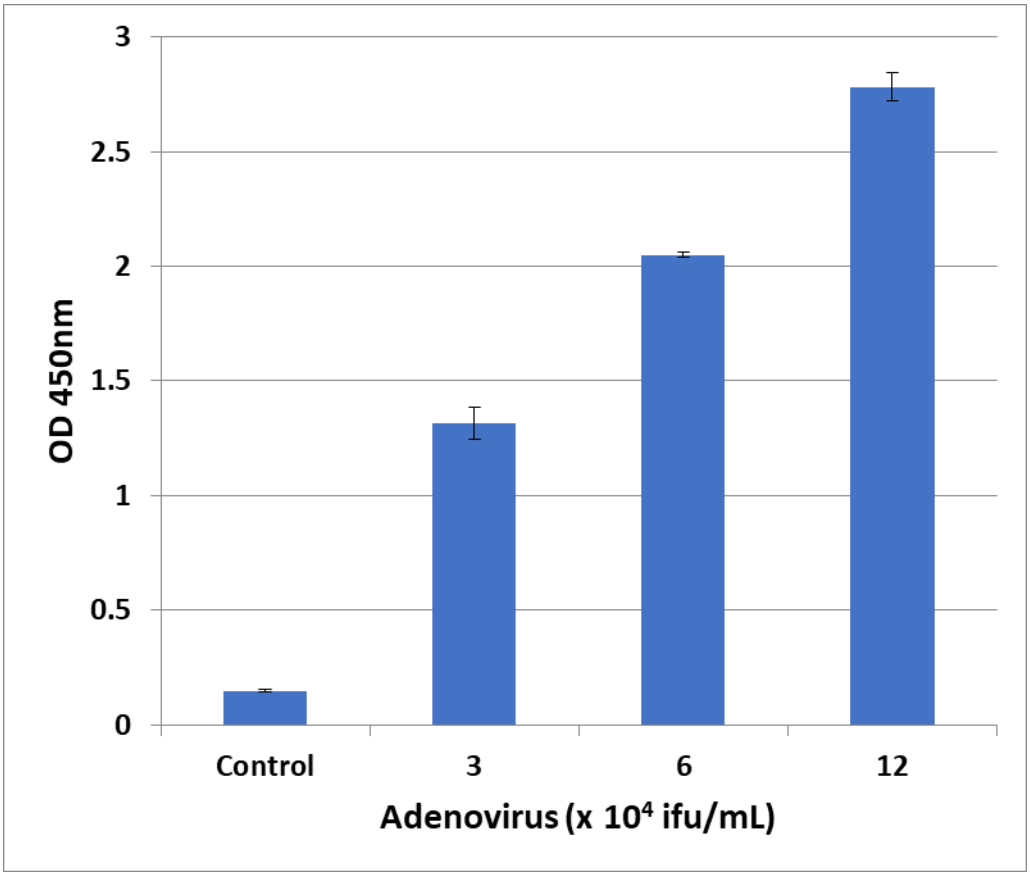

Figure 2: Hexon in Recombinant Adenovirus. 1.0 x 10^9 ifu/mL recombinant adenovirus was first diluted 8000-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. Adenoviral lysate was subjected to Adenovirus Hexon ELISA Kit according to Assay Protocol.

References

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Warranty

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