
Product Manual

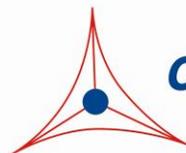
QuickTiter™ Adenovirus Quantitation Kit

Catalog Number

VPK-106

20 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages they provide when introducing genetic material into host cells. The permissive host cell range is very wide. The virus has been used to infect many mammalian cell types (both replicative and non-replicative) for high expression of the recombinant protein. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with liposome. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells.

HEK 293 cells or their variants are used as host cells for viral amplification. Recombinant adenoviruses can be grown at high titer (10^{10} VP (viral particles)/mL, which can be concentrated up to 10^{13} VP/mL) and purified by Cell Biolabs ViraBind™ Adenoviral Purification Kit or traditional CsCl ultracentrifugation.

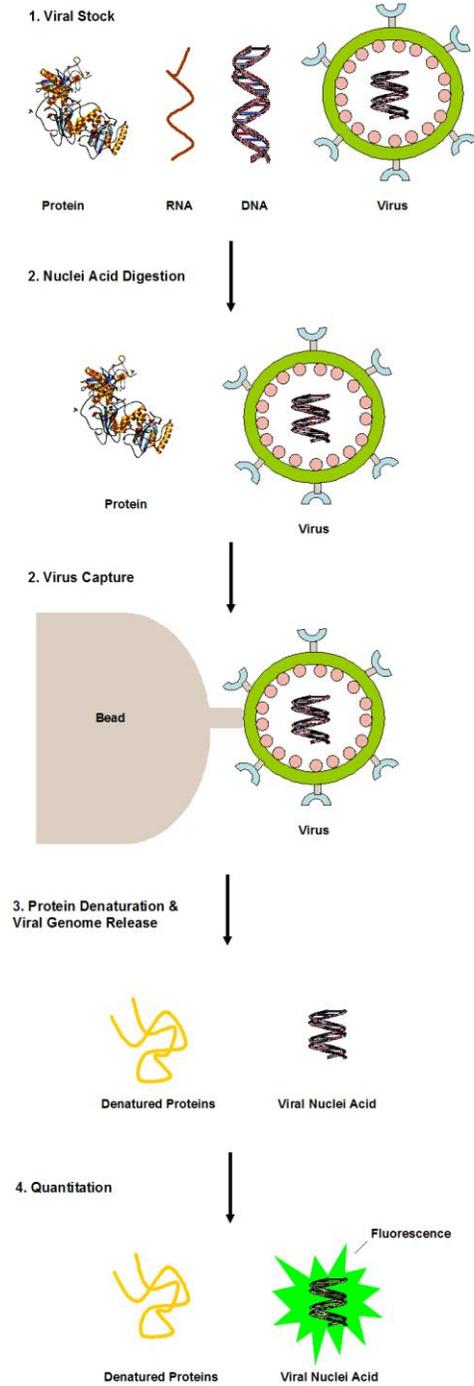
A particular challenge in the delivery of a gene by a viral vector is the accurate measurement of virus titer. Traditionally, infectivity particles are measured in culture by a plaque-forming unit assay (PFU) that scores the number of viral plaques as a function of dilution. Others utilize antibodies that recognize adenovirus hexon proteins by immunohistochemistry staining or FACS analysis. These methods are time-consuming, require a long infection period, and suffer from a high degree of inter-assay variability and are affected by virus-cell interactions. 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 some of the components it contains can contribute to the optical absorbance of 260 nm.

Cell Biolabs' proprietary QuickTiter™ Adenoviral Quantitation Kit does not involve cell infection; instead, it specifically measures the viral nucleic acid content of purified viruses or unpurified viral supernatant sample (See Test Principle). Especially for unpurified viral supernatant, the kit is very useful for determining the supernatant titer before the purification step. The kit has detection sensitivity limit of 1×10^9 VP or 1×10^{10} VP/mL when 100 μ L of adenoviral supernatant is used in the assay, which is sufficient for mid or high-titer adenovirus sample. The entire procedure takes about 45 to 60 minutes. Each kit provides sufficient quantities to perform up to 20 tests for viral samples and controls.

QuickTiter™ Adenoviral Quantitation Kit provides an efficient system for rapid quantitation of adenovirus titer for both viral supernatant and purified virus.

Assay Principle

How QuickTiter™ Kit Works*



* QuickTiter™ Method: patent pending

Related Products

1. AD-100: 293AD Cell Line
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent

3. VPK-099: ViraBind™ Adenovirus Miniprep Kit
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-110: QuickTiter™ Adenovirus Titer ELISA Kit
6. VPK-111: Rapid RCA Assay Kit
7. VPK-252: RAPAd® CMV Adenoviral Expression System

Kit Components (shipped on blue ice)

1. QuickTiter™ Solution A (Part No. 40020): One tube – 200 µL.
2. QuickTiter™ Adenovirus Capture Solution (Part No. 40021): One tube – 1.0 mL.
3. QuickTiter™ Solution B (10X) (Part No. 40022): Two tubes – 1.8 mL each.
4. QuickTiter™ Solution C (2X) (Part No. 40023): Two tubes – 1.5 mL each
5. CyQuant® GR Dye (400X) (Part No. 105101): One tube – 50 µL.
6. QuickTiter™ Adenovirus DNA Standard (Part No. 40025): One tube – 500µL containing 100 µg/mL Adenovirus DNA Standard

Materials Not Supplied

1. Recombinant adenovirus of interest: purified or unpurified high-titer virus supernatant
2. HEK 293 cells and cell culture growth medium
3. Cell culture centrifuge
4. 0.45 µm filter
5. 1X PBS containing 10 mM MgCl₂, 1 mM CaCl₂
6. 1X TE (10 mM Tris, pH 7.5, 1 mM EDTA)
7. Fluorescence Plate Reader

Storage

Store all kit components at 4°C.

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 QuickTiter™ Solution B: Prepare a 1X QuickTiter™ Solution B by diluting the provided 10X stock 1:10 in deionized water. Store the diluted solution at room temperature.
- 1X QuickTiter™ Solution C: Prepare a 1X QuickTiter™ Solution C by diluting the provided 2X stock 1:2 in deionized water. Store the diluted solution at room temperature.

- 1X CyQuant® GR Dye: Estimate the amount of 1X CyQuant® GR Dye needed based on the number of assays including adenovirus DNA standard samples. Immediately before use, prepare a 1X CyQuant® GR Dye by diluting the provided 400X stock 1:400 in 1X TE. For best results, the diluted solution should be used within 2 hrs of its preparation.

Preparation of Standard Curve

1. To create adenovirus DNA standards from 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL,... 0 µg/mL (1:2 serial dilution), label nine microcentrifuge tubes #1 to #9.
2. Add 20 µL of 1X QuickTiter™ Solution C to tube #2 to #9, transfer 20 µL of 100 µg/mL QuickTiter™ Adenovirus DNA Standard to tube #1 and #2. Mix tube #2 well, transfer 20 µL of the mixture (50 µg/mL) to the next tube. Repeat the steps through tube #8 and use tube #9 as a blank.
3. Transfer 5 µL of each dilution including blank to a microtiter plate suitable for fluorometer. Add 95 µL of 1X CyQuant® GR Dye to each of the wells containing the 5 µL sample. Read the plate with a fluorescence plate reader using a 480/520 nm filter set.

Harvesting Infected Cell Lysate

The following procedure is suggested for a T75 flask and may be optimized to suit individual needs.

1. Use HEK 293 cells that have been passaged regularly 2-3 times prior to the infection. Culture these cells until the monolayer is 90-100% confluent.
2. Replace the cell culture media with new growth media, 15 mL per flask. Next, the adenovirus is added to the culture. Either crude or purified viral stock can be used. A multiplicity of 10 PFU (plaque forming units) per cell is desired.
3. After 24 hr, some cells should be floating. Add 10 mL growth media to the culture flask and allow the viruses to expand for another 24 hrs. When all the cells are floating, gently shake the culture flask several times and harvest all media, including cells, in a sterile tube.
4. Release the adenoviruses from the cells with three freeze/thaw cycles. Centrifuge at 3000 rpm for 10 minutes to pellet the cell debris. Discard the pellet and save supernatant. If a large amount of cell debris is still visible, centrifuge the supernatant again.
5. The viral supernatant can be stored at -80°C or immediately used in the titration step.

Assay Protocol

1. When unpurified viral supernatant is used, the supernatant is clarified by passing it through a 0.45 µm sterile filter before proceed the next step.
2. Add viral sample (1 to 100 µL) to a 1.5 mL microcentrifuge tube and adjust the final volume to 1 mL with 1X PBS containing 10 mM MgCl₂, 1 mM CaCl₂.

Note: A proper negative control MUST be included. For purified viral sample, use the same volume of buffer solution that viruses are stored. For unpurified viral supernatant, use the same volume of uninfected 293 cell lysate supernatant that has been through three freeze/thaw cycles.

3. Add 10 µl of QuickTiter™ Solution A to the assay tube and mix by inverting the tube several times. Incubate at 37°C for 30 minutes.

4. Mix the QuickTiter™ Adenovirus Capture Solution by vortexing for 10 seconds. Quickly transfer 40 µL of the bead capture solution to the assay tube containing the viral sample. Incubate at room temperature for 10 min on an orbital shaker.
5. Spin down the beads at 2000X g for 30 seconds. Discard the supernatant and wash the beads with 750 µL of 1X QuickTiter™ Solution B. Mix by inverting the tube several times, spin down the beads and discard the supernatant.
6. Repeat the wash step once and aspirate the final wash. To remove the last bit of liquid, centrifuge the tube again at 2000X g for 30 seconds, and remove remaining supernatant with a small-bore pipette tip to avoid the beads.
7. Add 20 µL of 1X QuickTiter™ Solution C, mix with the beads by vortexing for 10 seconds, spin down the beads at 12000g for 30 seconds.
8. Transfer 5 µL supernatant to a microtiter plate suitable for fluorometer. Add 95 µL of freshly prepared 1X CyQuant® GR Dye to well(s) containing the 5 µL supernatant. Read the plate with a fluorescence plate reader using a 480/520 nm filter set.
9. Calculate adenovirus virus titer based on the standard curve.

Example of Results

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

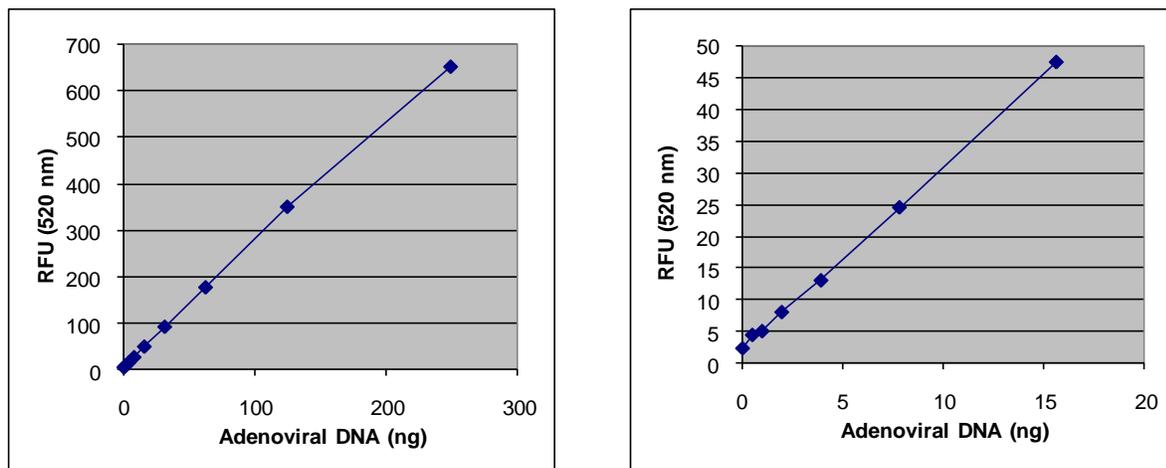


Figure 1: Adenovirus DNA Standard Curve. The QuickTiter™ Adenovirus DNA Standard was diluted as described in the above instructions. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Device) with a 485/538 nm filter set and 530 nm cutoff.

Calculation of Adenovirus Titer (VP/mL)

1. Determine Viral DNA amount:

- 1) Calculate Net RFU (Relative Fluorescence Unit):

$$\text{Net RFU} = \text{RFU (viral sample)} - \text{RFU (negative control corresponding to viral sample)}$$
- 2) Use the standard curve to determine the viral DNA amount of each unknown sample.

2. Calculate Viral Titer:

The average genome size of an adenovirus is 40 kbp, therefore,

1 ng adenoviral DNA = $(1 \times 10^{-9}) \text{ g} / (40,000 \text{ bp} \times 660 \text{ g/bp}) \times 6 \times 10^{23} = 2.3 \times 10^7 \text{ VP}$

Virus Titer (VP/mL) = $\frac{\text{Amount of adenoviral ds DNA (ng)} \times 2.3 \times 10^7 \text{ VP} \times (20 \mu\text{L}/5 \mu\text{L})}{\text{Viral sample volume (mL)}}$

Virus Titer (VP/mL) = $\frac{\text{Amount of adenoviral ds DNA (ng)} \times 9.2 \times 10^7 \text{ VP/ng}}{\text{Viral sample volume (mL)}}$

Examples of Ad-β Gal Titer Quantitation:

Method: HEK 293 cells were infected with Ad-β Gal at 20 MOI for 48 hrs. After three freeze/thaw cycles to release ad-β Gal viruses from infected cells, 20 mL of viral supernatant was collected and filtered through a 0.45 μm sterile filter. Ad-β Gal was further purified using our ViraBind™ Kit or CsCl₂ ultracentrifugation. The adenovirus titers were determined as described in assay instructions.

Sample #1: Ad-β Gal Viral Supernatant: 100 μL was used

Average Net RFU = 165.7 – 22.3 = 143.4 or 51 ng of viral DNA

Virus Titer (VP/mL) = $\frac{51 \text{ (ng)} \times 9.2 \times 10^7 \text{ VP/ng}}{0.1 \text{ mL}} = 4.7 \times 10^{10} \text{ VP/mL}$

Sample #2: Purified Ad-β Gal (ViraBind™ Kit): 50 μL was used

Average RFU = 529.4 – 3.7 = 525.7 or 197 ng viral DNA

Virus Titer (VP/mL) = $\frac{197 \text{ (ng)} \times 9.2 \times 10^7 \text{ VP/ng}}{0.05 \text{ mL}} = 3.6 \times 10^{11} \text{ VP/mL}$

Sample #3: Purified Ad-β Gal (CsCl₂): 2 μL was used

Average RFU = 64 – 4.0 = 60 or 22 ng viral DNA

Virus Titer (VP/mL) = $\frac{22 \text{ (ng)} \times 9.2 \times 10^7 \text{ VP/ng}}{0.002 \text{ mL}} = 1.0 \times 10^{12} \text{ VP/mL}$

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Recent Product Citations

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