Product Manual

QuickTiter™ Adenovirus Titer ELISA Kit

Catalog Number

VPK- 110 **2 x 96 wells**

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. These methods are time-consuming (10 days), require a long infection period, and suffer from a high degree of inter-assay variability and are affected by virus-cell interactions. Cell Biolabs QuickTiter™ Adenovirus Titer ELISA Kit utilizes an antibody against adenovirus hexon proteins to quantitate infected cells. The hexon proteins are the largest and most abundant of the structural proteins in the adenovirus capsid, and they are distributed symmetrically to form capsid facets.

Cell Biolabs QuickTiter™ Adenovirus Titer ELISA Kit provides a quick and complete system to functionally titer virus infectivity; it provides sufficient reagents for up to 192 tests in 96-well plates. In contrast to the 10-day infection of a classical plaque assay, the kit only requires a 2-day infection. Detection sensitivity is 10^4 ifu/mL (10^6-10^7 VP/mL), which is sufficient for most adenoviral samples. The kit recognizes all 41 serotypes of adenovirus by immunocytochemistry and can be used with any adenovirus system as long as the virus is able to amplify in HEK 293 cells.

Related Products

1. VPK-106: QuickTiter™ Adenovirus Quantitation Kit
2. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
3. VPK-111: Rapid RCA Assay Kit
4. VPK-252: RAPAd® CMV Adenoviral Expression System
5. AD-100: 293AD Cell Line
6. VPK-099: ViraBind™ Adenovirus Miniprep Kit
7. VPK-100: ViraBind™ Adenovirus Purification Kit
8. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
9. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
10. VPK-120: QuickTiter™ Retrovirus Quantitation Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Hexon Antibody (Part No. 10901): One tube – 30 µL.
2. Secondary Antibody, HRP Conjugate (Part No. 10902): One tube – 50 µL.
3. TMB Substrate (Part No. 211002): One amber bottle – 20 mL.
4. Stop Solution (Part No. 211003): One bottle – 20 mL.

Box 2 (shipped on blue ice packs)

1. Ad-β gal Positive Control (Part No. 10904): One tube – 50 µL at 1.0 x 10^9 ifu/mL.

Materials Not Supplied

1. Recombinant adenovirus of interest
2. HEK 293 cells and cell culture growth medium
3. Methanol
4. 1% BSA/PBS
5. Wash Buffer such as PBS
6. Microtiter plate reader

Storage

Upon receipt, store the Ad-β gal Positive Control at -80ºC. Store all other kit components at 4ºC.

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 Anti-Hexon antibody solution: Prepare a 1X anti-hexon antibody solution by diluting the provided Anti-Hexon antibody stock 1:1000 in 1% BSA/PBS. Store the diluted solution on ice.
- 1X Secondary antibody solution: Prepare a 1X Secondary antibody solution by diluting the provided stock 1:2000 in 1% BSA/PBS. Store the diluted solution on ice.

Preparation of Standard Curve

1. Immediately before infection, create a 2-fold serial dilution of Ad-β gal positive control in culture medium. First, dilute original viral stock 1:2000. For example, add 4 µL of viral sample to a sterile 15 ml conical tube containing 8 mL of culture medium.

2. Label eight sterile tubes #1 to #8, and add 500 µL of culture medium to each tube. Add 500 µL of the 1:2000 diluted Ad-β gal viral sample (from step 1) to tube #1 and mix well. Transfer 500 µL of the tube #1 mixture (2.5×10^5 ifu/mL) to the next tube. Repeat the steps through tube #7 and use tube #8 as a blank.

Preparation of Adenoviral Samples

- For unknown viral samples, create 10-fold serial dilutions with culture medium. For example, start with a 1:100 dilution of the original viral samples by adding 10 µL of viral sample to a sterile tube containing 990 µL of culture medium. Transfer 100 µL of the mixture to the next tube containing 900 µL of culture medium. Repeat this step several times. For accurate assessment of viral titer, one of the dilutions for your unknown viral sample should be within the range of the Ad-β gal standard curve (4.0×10^3 ifu/mL to 2.5×10^5 ifu/mL).

Assay Protocol

I. Virus Infection

1. Harvest HEK 293 cells and resuspend cells in culture medium at 5×10^5 cells/mL. Seed 100 µL in each well of a 96-well plate and incubate at 37ºC, 5% CO_2 for 1 hr.

   Note: Adenovirus titer assay is critically dependent on the firm attachment of cells. If the cells look thin and easy to come off during immunostaining steps, you won't get consistent results. Only use low passage 293 cells with flattened morphology or 293AD (Cat. # AD-100), a selected 293 cell line for plasmid transfection, adenovirus amplification and titering. To improve cell adhesion, you can also precoat the plate with polylysine or extracellular matrix.

2. Prepare serial dilutions of the Ad-β gal positive control and your viral sample in culture medium. Dropwise add 50 µL of diluted viral sample to each well of the 96-well assay plate (note: a negative control should be performed simultaneously). To ensure accuracy, perform each sample in duplicate.
3. Incubate infected cells at 37ºC, 5% CO_2 for 2 days.

II. Immunoassay

1. Slowly remove medium from the wells by tilting the plate and aspirating from the edge, then fix infected 293 cells by gently adding 100 µL of cold methanol down the side of each well of the 96-well assay plate, taking care not to dislodge the cells. Incubate 20 minutes at -20ºC.
2. Gently wash the fixed cells three times with 1X PBS, five minutes each wash.
3. Block for 1 hr with 200 µL of 1% BSA in PBS per well at room temperature on an orbital shaker.
4. Add 100 µL of diluted 1X anti-Hexon antibody solution to each well and incubate for 1 hr at room temperature on an orbital shaker.
5. Gently wash the fixed cells three times with 1X PBS, five minutes each wash.
6. Add 100 µL of diluted 1X Secondary antibody solution (HRP-conjugated) to each well and incubate for 1 hr at room temperature on an orbital shaker.
7. Gently wash the fixed cells five times with 1X PBS, five minutes each wash.

8. Warm TMB Substrate to room temperature. Add 100 µL of TMB Substrate solution and incubate at room temperature for 5 to 10 minutes. Stop reaction by adding 100 µL of Stop Solution to each well.
9. Measure Optical Density at 450nm on a 96-well plate reader. Calculate the viral titer based on the standard curve from Ad-β gal positive control titrations.

Example of Results

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

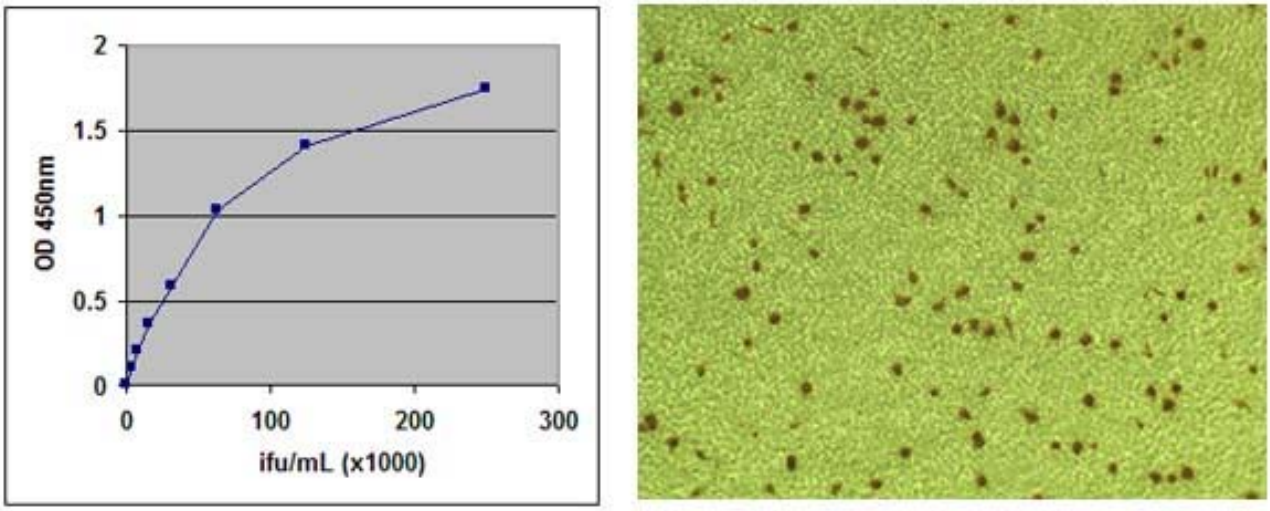

Figure 1: Ad-β gal Titration. Serial dilutions of the Ad-β gal positive control were used to infect HEK 293 cells for 48 hours. Left panel: Anti-Hexon ELISA was performed according to the assay protocol. Right panel: Adenovirus-infected 293 cells were visualized by anti-Hexon immunocytochemistry staining using QuickTiter™ Adenovirus Immunoassay Kit (#VPK-109). The picture was taken from 293 cells infected with 10^5 ifu/mL of Ad-β gal in a 12-well plate.

References

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Recent Product Citations

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Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com