Product Manual

QuickTiter™ Wild Type Adenovirus Titer Immunoassay Kit

Catalog Number

VPK-5168 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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.

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 (10-14 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™ Immunoassay Kit utilizes an antibody against adenoviral hexon proteins to visualize infected cells by immunocytochemistry staining. 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™ Immunoassay Kit provides a quick and complete system to functionally titer virus infectivity. The kit provides sufficient reagents for up to 100 titrations in a 24-well plate. In contrast to the 10 to 14-day infection of a classical plaque assay, the kit only requires a 2-day infection. The kit antibody against adenoviral hexon protein recognizes all 51 serotypes of adenovirus by immunocytochemistry and can be used with any adenovirus system as long as the virus is able to amplify in A549 or similar permissive cells.

Assay Principle

Seed A549 cells in a 24-well plate

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Prepare Adenovirus Dilutions and Infect A549 cells for 48 hours

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ICC Staining with Anti-Adeno Antibody

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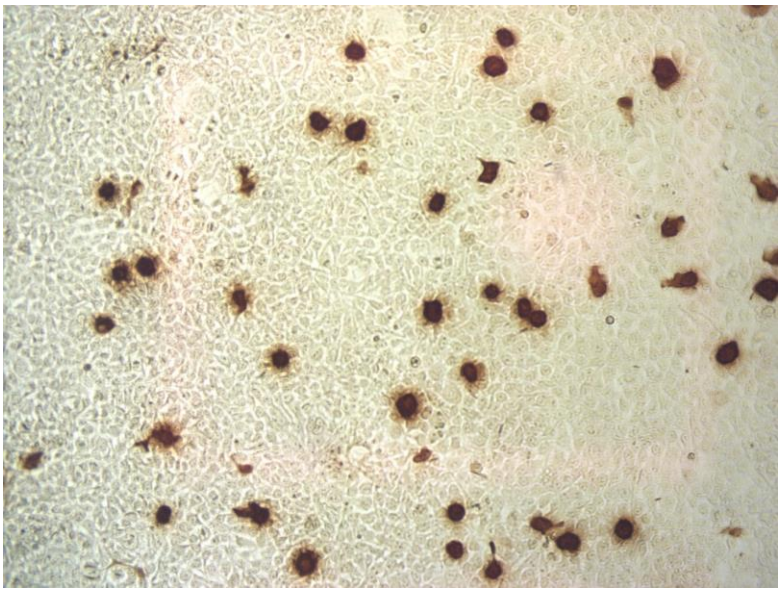
Count Positive Cells and Calculate Viral Titer



Related Products

1. VPK-252: RAPAd® CMV Adenoviral Expression System
2. VPK-099: ViraBind™ Adenovirus Miniprep Kit

Kit Components (shipped on blue ice)

1. Anti-Adenovirus Antibody (1000X) (Part No. 51681C): One 30 µL tube.
2. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902): One 50 µL tube.
3. DAB Substrate (25X) (Part No. 10903): One 1.5 mL tube.
4. Diluent (10X) (Part No. 10905): One 4.5 mL bottle.

Materials Not Supplied

1. Adenovirus of interest
2. A549 cells and cell culture growth medium
3. Methanol
4. 1% BSA/PBS
5. H_2O_2
6. Light Microscope

Storage

Upon receipt, store all 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

The table below is suggested for tests in a 24-well plate. Use twice the amount of reagents for samples in a 12-well plate.

- 1X Anti-Adenovirus antibody solution: Prepare a 1X Anti-Adenovirus antibody solution by diluting the provided 1000X Anti-Adenovirus 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 1000X stock 1:1000 in 1% BSA/PBS. Store the diluted solution on ice.
- 1X DAB working solution: Prior to use, FRESHLY prepare a 1X DAB working solution. First dilute the provided 10X Diluent to 1X with ddH_2O, and add H_2O_2 to a final concentration of 0.01%. Then dilute the 25X DAB stock to 1X with 1X Diluent/ H_2O_2 and use the 1X DAB working solution immediately.

  Note: Dilute 10X diluent using ddH_2O. Heavy metals in impure H_2O will cause DAB precipitation.

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Reagents	24 tests (24-well plate)	48 tests (24-well plate)	96 tests (24-well plate)
1000X Anti-Adenovirus Antibody	6 µL	12 µL	24 µL
1000X Secondary Antibody	6 µL	12 µL	24 µL
25X DAB	240 µL	480 µL	960 µL
Final Volume (Each Reagent)	**6 mL**	**12 mL**	**24 mL**

Table 1. Preparation of Antibody and DAB solutions.

Preparation of Adenoviral Samples

1. Immediately before infection, create a 10-fold serial dilution of viral sample from 10^{-3} to 10^{-7}. First, dilute original viral sample 1:100. For example, adding 10 µL of viral sample to a sterile tube containing 990 µL of culture medium.
2. Label six sterile tubes #1 to #6, and add 900 µL of culture medium to each tube. Add 100 µL of 1:100 diluted viral sample to tube #1, mix tube #1 well. Transfer 100 µL of the mixture (1:1000 dilution) to the next tube. Repeat the steps until tube #5 and use tube #6 as a blank.

Assay Protocol

The instructions below are suggested for assays performed in 24-well plate. Use twice as much the amount of cells and reagents for assays performed in 12-well plate.

I. Virus Infection

1. Harvest A549 cells and resuspend cells in culture medium at 2.5 x 10^5 cells/mL. Seed 1 mL in each well of a 24-well plate and incubate at 37ºC, 5% CO_2 for 1 hr.

   Note: Adenovirus titer assays are critically dependent on the firm attachment of cells. To improve cell adhesion, you can precoat the plate with polylysine or extracellular matrix.

2. Prepare a 10-fold serial dilution of your viral sample in culture medium. Dropwise add 100 µL of diluted viral sample to each well of the 24-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. Immunostaining

1. Slowly remove medium from the wells by tilting the plate and aspirating from the edge, then fix infected A549 cells by gently adding 0.5 ml of cold methanol down the side of each well of the 24-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 1% BSA in PBS at room temperature on an orbital shaker.
4. Add 0.25 mL of diluted 1X Anti-Adeno 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 0.25 mL 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. Add 0.25 mL of freshly diluted 1X DAB working solution to each well and incubate for 10 minutes at room temperature on an orbital shaker.

   Note: Adenovirus infected cells should show dark brown staining within 5 minutes. During incubation, excess DAB starts to form light precipitates in solution, and this will not affect the staining results.
9. Aspirate DAB, wash twice with 1X PBS and add 1 mL of 1X PBS to each well.
10. Count positive stained cells (brown) for at least five separate fields per well using a light microscope and 10X objective.
11. Calculate the average number of positive cells per well and viral titer (infectious units/mL).

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.

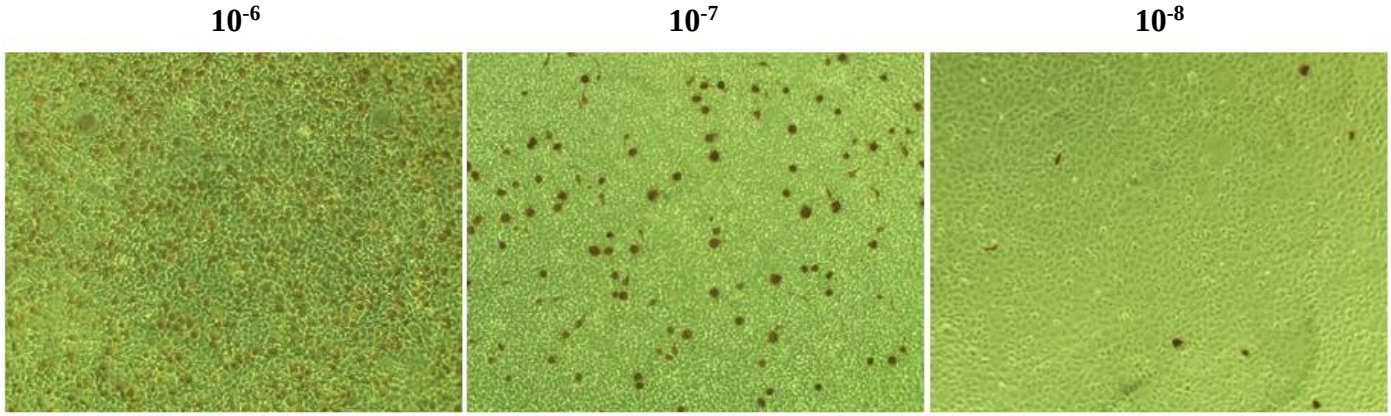

Figure 1: Wild Type Ad5 Titration. Serial 10-fold dilutions of purified wild type Ad5 were used to infect A549 cells in 24-well plate for 48 hrs. Anti-Adeno immunostaining was performed as described in the Assay Protocol.

Calculation of Adenovirus Titer (Infectious Units/mL)

1. Calculate the average number of positive cells per field. Ideally, choose a dilution with 5-50 positive cells/field and count at least five fields.
2. Determine the number of fields per well

   For most microscopes, a standard 10X objective lens with 10X eyepiece lens has a field diameter (D) of 1.8 mm, then:

   Area per field = $3.14 \times (D/2)^2 = 3.14 \times 0.9^2 = 2.54\ mm^2$

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For 24-well plate, area of a well (standard 24-well plate) is 2.0 cm^2, therefore,
Fields/well = 2.0 cm^2/2.54 mm^2 = 2.0 cm^2/2.54 x 10^{-2} cm^2 = **79**

For 12-well plate, area of a well (standard 12-well plate) is 3.8 cm^2, therefore,
Fields/well = 3.8 cm^2/2.54 mm^2 = 3.8 cm^2/2.54 x 10^{-2} cm^2 = **150**

Note: If you are not sure about the field diameter of the 10X objective lens you are using or you are using objective lenses other than 10X, the field diameter can be determined by aligning with the grids of hemacytometer (Figure 2), or referring to Table 2.

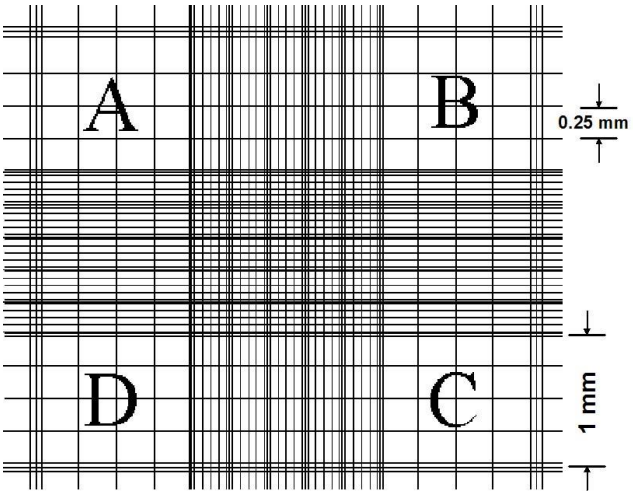

Figure 2. Hemacytometer Grid Dimensions.

Objective Lenses	Eyepiece Lenses (10X)			Fields/Well	
	Total Magnification	Field Diameter	Field Area (mm^2)	12-well Plate	24-well Plate
4X	40X	5 mm	19.6	19	10
10X	100X	1.8 mm	2.54	150	79
20X	200X	0.9 mm	0.64	594	313

Table 2. Field sizes of objective lenses.

3. Calculate viral titer (Infectious Units or ifu/mL)

Tests in 24-well:

$$\text{Viral Titer (ifu/mL)} = \frac{\text{(average positive cells/field) x (79 fields/well) x (dilution factor)}}{\text{(0.1 mL)}}$$

Tests in 12-well:

$$\text{Viral Titer (ifu/mL)} = \frac{\text{(average positive cells/field) x (150 fields/well) x (dilution factor)}}{\text{(0.1 mL)}}$$

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Calculation Example:

A series of 10-fold dilutions of wild type Ad5 was made and the titer was determined in a 24-well plate as described in assay instruction. Ten fields were counted and the average positive cells/field was 7 for $1/10^7$ dilution under a standard 10X objective, therefore:

$$\text{Viral Titer (ifu/mL)} = \frac{\text{(average positive cells/field) x (79 fields/well) x (dilution factor)}}{\text{(0.1 mL)}}$$

$$\text{Viral Titer (ifu/mL)} = \frac{\text{(7/field) x (79 fields/well) x } (10^7)}{\text{(0.1 mL)}} = 5.5 \times 10^{10} \text{ (ifu/mL)}$$

References

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Warranty

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