Product Manual

Viral Plaque Assay Kit

Catalog Numbers

VPK-5191 120 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Developed in 1952, the plaque assay was the first method for quantifying viral concentrations. Advanced by Renato Dulbecco, the assay allows for the number of plaque-forming units (PFU) to be manually determined in a given sample containing replication-competent lytic virions.

Plaque assays require cultured cells susceptible to infection by the virus of interest. The cells are first seeded onto a surface they can adhere to and grow on, then left overnight to form a confluent monolayer. A virus sample is then diluted several times, and an aliquot of each dilution is added to a dish or well of cells. An incubation period allows the virus to attach to target cells before removing the inoculum. The culture is then covered with a medium containing nutrients, plus a substance, such as agarose or methylcellulose, that forms a gel or semisolid overlay. Infectious virus particles that enter cells and replicate can then trigger the release of progeny virions. The gel restricts particle movement so that newly produced viruses can only infect neighboring cells. When the virus kills infected cells, the dead (or dying) cells detach and create a hole in the cell monolayer. This area devoid of cells is called a plaque and appears as circular spots on the growth surface.

Cell Biolabs' Viral Plaque Assay Kit provides an accurate system to functionally titer lytic virus infectivity. The kit provides sufficient reagents for up to 120 titrations in a 12-well plate.

Assay Principle

Day 1: Seed Cells in a 12-well or 6-well Plate

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Day 2: Infect Cells with Serial Dilutions of Virus of Interest and Add Overlay Media

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Monitor Plaque Development under a Light Microscope

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Day 4-16: Fix and Stain Cells

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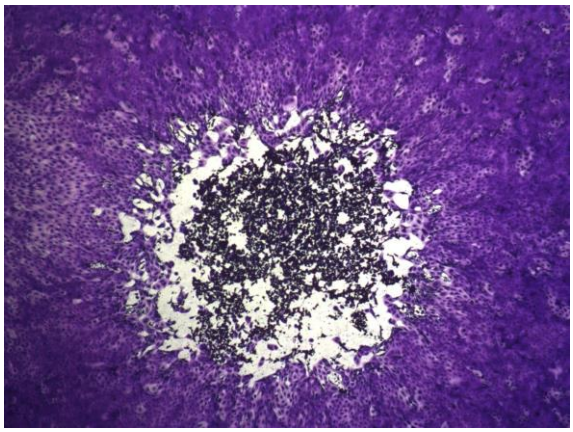
Count Positive Plaques and Calculate Viral Titer



Related Products

1. VPK-5170: RSV Fusion Protein ELISA Kit
2. VPK-5174: Influenza A Nucleoprotein ELISA Kit
3. VPK-5175: Influenza B Nucleoprotein ELISA Kit

4. VPK-5189: Influenza A Immunoplaque Assay Kit
5. VPK-5190: Influenza B Immunoplaque Assay Kit

Kit Components

1. CytoSelect™ Agar Powder (Part No. 113001): One 1.2 g bottle
2. 10X Fixation Solution (Part No. 51911A): One 20 mL bottle
3. Cell Stain Solution (Part No. 51912A): One 30 mL bottle

Materials Not Supplied

1. Cells and Virus of Interest
2. 2X Cell Culture Medium and sterile DPBS
3. Sterile cell culture grade water
4. Microwave or Heating Block
5. Water Bath
6. Light Microscope

Storage

Store all kit components at room temperature.

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 Fixation Solution: Prepare a 1X Fixation solution by diluting the provided 10X Fixation Solution 1:10 in PBS. Store the diluted solution at room temperature.

Preparation of Overlay Media

1. Preparation of 1% Agar Solution: Place 1.0 g of Agar Powder in a sterile bottle, add 100 mL of sterile cell culture grade water. Autoclave or microwave until agar is completely dissolved. Aliquot and store the 1% Agar Solution at 4 ºC.
2. Melt 1% Agar Solution in a 56ºC water bath or microwave, and then cool to 37ºC in a water bath.
3. Warm the 2X plaque medium to 37ºC in a water bath. Allow at least 30 minutes for the temperature to equilibrate.

   Note: Not all plaque media contain the same ingredients as host cell culture media. For example, fetal bovine serum (FBS) is omitted in Influenza viral plaque assay medium, since it can inhibit Influenza propagation through inhibition of certain proteases which are required for viral fusion.

TPCK-Trypsin is usually added to the plaque medium to facilitate Influenza viral fusion and entry of the host cells.

4. According to Table 1 (below), prepare the desired volume of Overlay Media by mixing warmed 2X cell culture medium and 1% Agar Solution at 1:1 ratio. Maintain the Overlay Media at 37ºC to avoid gelation.

	12-well Plate	6-well Plate or 35 mm Dish	60 mm Dish
Cells/Well	0.5×10^6	1.0×10^6	2.0×10^6
Viral Inoculum (µL)	200	400	800
Overlay Medium (mL)	1.5	3	6
1X Fixation Solution (mL)	1.5	3	6
Cell Stain Solution (mL)	0.25	0.5	1.0

Table 1: Reagent amounts required for different plate/dish formats.

Assay Protocol

The instructions below are suggested for assays performed in a 12-well plate. For other sizes of plates or dishes, use the amount of each reagent listed in Table 1 (above).

I. Virus Infection

1. Harvest cells and resuspend cells in culture medium at 2.5×10^5 cells/mL. Seed 2 mL in each well of a 12-well plate and incubate at 37ºC, 5% CO_2 overnight or until 90-100% confluence.
2. Prepare a 5- or 10-fold serial dilution of your viral sample in culture medium. Rinse cells once with DPBS, and add 200 µL of diluted viral sample to each well of the 12-well assay plate. Each sample should be performed in duplicate, and a negative control should be performed simultaneously.
3. Incubate infected cells at 37ºC, 5% CO_2 for 45-60 minutes. Gently rock the plate every 10 minutes to ensure even coverage and prevent the cell monolayer from drying.
4. Aspirate the viral inocula and wash once with DPBS.
5. Gently add 1.5 mL of warmed Overlay Media, and allow the plate to sit at room temperature for 20 minutes until the Overlay Media solidifies.
6. Incubate the infected cells at 37ºC, 5% CO_2 for 2-14 days or until the plaques can be visualized under a light microscope. Add fresh Overlay Media as needed.

II. Cell staining

1. Add 1.5 mL of 1X Fixation Solution directly on top of the Overlay Media to fix the cells and inactivate the virus. Incubate 1 hr to overnight at room temperature on an orbital shaker.
2. Aspirate the fixation solution and remove the semisolid Overlay Media with slowly running water. Gently wash the fixed cells two times with 1X PBS.
3. Add 0.25 mL of Cell Staining Solution to each well and incubate for 30 min at room temperature on an orbital shaker.
4. Gently wash the stained cells with water, and dry the plate.
5. Count positive stained plaques (brown).

Calculation of Virus Titer (pfu/mL)

Count the plaques in each well. Disregard wells with fewer than 5 or greater than 100 plaques. The negative control should have a uniform monolayer and can be used as a reference control.

Tests in a 12-well plate:

$$\text{Viral Titer (pfu/mL)} = \frac{\text{(average positive plaques per well) x (dilution factor)}}{\text{(0.2 mL)}}$$

Tests in a 6-well plate or 35 mm dish:

$$\text{Viral Titer (pfu/mL)} = \frac{\text{(average positive plaques per well) x (dilution factor)}}{\text{(0.4 mL)}}$$

Tests in a 60 mm dish:

$$\text{Viral Titer (pfu/mL)} = \frac{\text{(average positive plaques per well) x (dilution factor)}}{\text{(0.8 mL)}}$$

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.

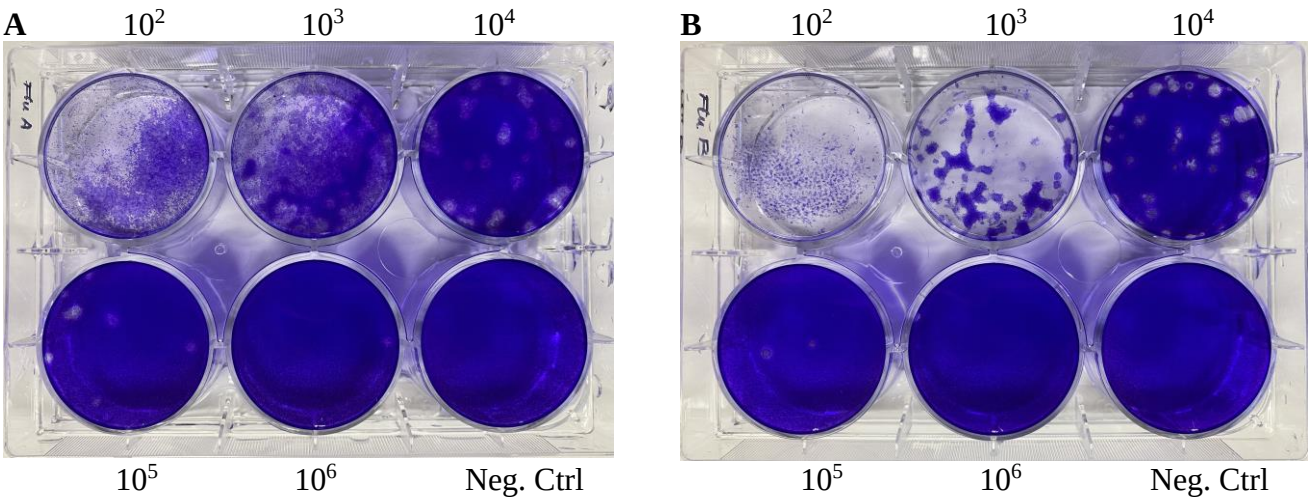


Figure 2: Influenza A and B Virus Plaque Assays in MDCK Cells. Different dilutions of Influenza A (Panel A) and B (Panel B) Virus Culture Fluid were used to infect MDCK cells for 72 hrs.

References

1. Baer, A. & Kehn-Hall, K. (2014) Viral concentration determination through plaque assays: using traditional and novel overlay systems. *J. Vis. Exp.* **93**, 52065.
2. Wen, Z. et al. (2019) Development and application of a higher throughput RSV plaque assay by immunofluorescent imaging. *J. Virol. Methods* **263**, 88–95.
3. Mendoza, E. J., Manguiat, K., Wood, H. & Drebot, M. (2020) Two detailed plaque assay protocols for the quantification of infectious SARS-CoV-2. *Curr. Protoc. Microbiol.* **57**, cpmc105.
4. Blaho, J. A., Morton, E. R. & Yedowitz, J. C. (2006) Herpes simplex virus: propagation, quantification, and storage. *Curr. Protoc. Microbiol.* **14**, E.1.1–E.1.23.
5. Cruz, D. J. M. & Shin, H.-J. (2007) Application of a focus formation assay for detection and titration of porcine epidemic diarrhea virus. *J. Virol. Methods* **145**, 56–61.

Warranty

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