Application Brief

Virology

Agilent

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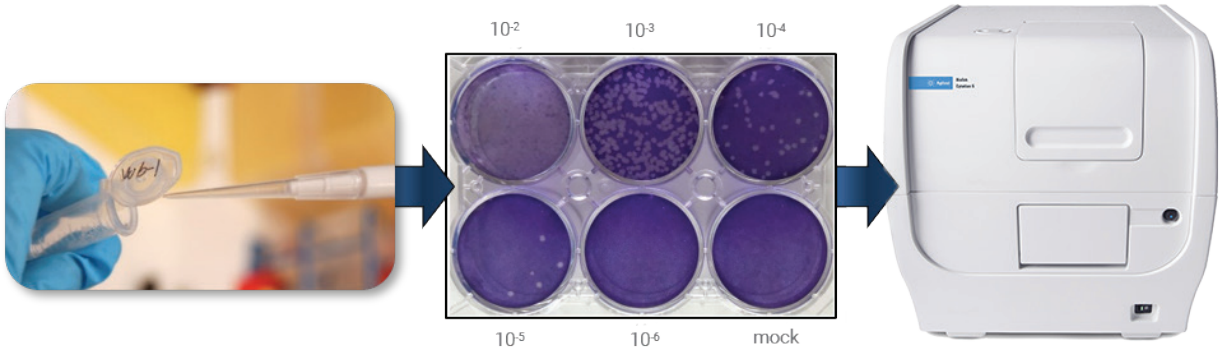
Automated Viral Plaque Assay Workflow Using the Cytation Cell Imaging Multimode Reader

Introduction

Plaque assays remain the standard method for determining viral titers for lysogenic viruses. The assay relies on determining the number of plaque-forming units (PFU) created in a monolayer of virus-infected cells. Plaques form when a virus-infected cell lyses, leading to subsequent cycles of infection and lysis of neighboring cells. The resulting cellular dead zone, or plaque, surrounded by non-infected cells is identified using a counterstain, typically a crystal violet solution, and optical microscopy or visual inspection. Plaques may take up to several days to form depending on the virus and are conventionally counted manually after an empirically determined amount of time. At appropriate dilutions, each plaque represents infection and lysis from a single virion and is used along with the dilution factor to calculate the number of plaque-forming units per sample volume (PFU/mL).

The practiced execution of the steps outlined above enable distinct counts of individual plaques to be achieved. However, the manual counting of plaques can be time intensive and subjective. This application brief describes an automated imaging-based plaque assay using the Agilent BioTek Cytation cell imaging multimode reader and Agilent BioTek Gen5 microplate reader and imager software. The assay provides an accurate and efficient method for determining plaque counts and calculating viral titers in a range of microplate densities, including 6-, 24-, 48-, and 96-well formats.



Monolayers infected with serial dilutions of virus - an agarose plug prevents coalescence of plaques.

Following incubation period and fixation, monolayer is counterstained for plaque visualization.

Viral titer determined by automated imaging and analysis with the Agilent BioTek Cytation 5 system.

Figure 1. Viral plaque assay workflow incorporating automated imaging and analysis conducted with the Agilent BioTek Cytation 5 cell imaging multimode reader and Agilent BioTek Gen5 microplate reader and imager software.

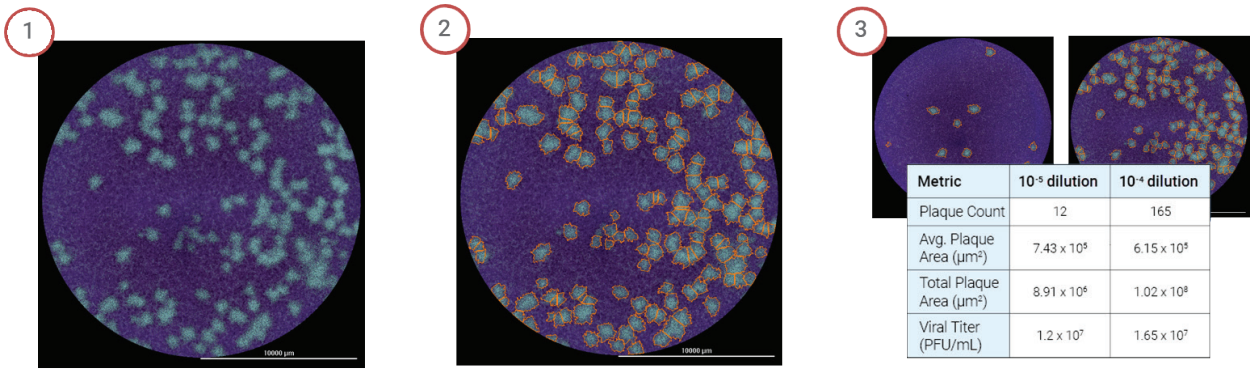
Experimental

Plaque assay materials and methods

BHK cell suspension was added to 6-well microplates to form a fully confluent and evenly distributed monolayer in each well. After 24 hours, spent media was removed and cells were washed with phosphate-buffered saline (PBS). Serial 1:10 dilutions of vesicular stomatitis virus (VSV) were then added to individual wells across the plate and incubated for adsorption. After 1 hour, the inoculum was removed and the cells were washed with basal medium. An agarose semisolid overlay was applied to the wells to prevent the coalescence of forming plaques, and the plate was incubated for 24 hours to allow for quantifiable plaque formation. Following incubation, cell monolayers were fixed with 4% paraformaldehyde (PFE) for 1 hour at room temperature. After fixation, the agarose overlay was carefully removed to prevent monolayer disruption. Crystal violet solution was added to each well for 15 minutes, followed by 2-4 gentle washes with distilled water.

Imaging procedure and analysis

Using the Cytation and Gen5 software, plaque counting, and calculation of final titers was determined using optimized protocols. Color images were captured using color brightfield and the 1.25x objective. An image montage was acquired per well to capture the entire culture area and then images were stitched together to form a single image for analysis. Individual plaques were identified by Gen5 automated analysis tools using the green color channel, based on size and intensity thresholding, with total plaque count, average plaque area, and total plaque area per well reported.



Capture
Color brightfield images of each prepared sample are captured then stitched and processed by automated Agilent BioTek Gen5 imaging protocol.

Analyze
Plaques are identified (orange mask) using optimized Agilent BioTek Gen5 cellular analysis tools.

Report
Plaque count, average plaque area, total plaque area, and viral titer are calculated and reported for comparison across samples.

Figure 2. Automated image acquisition and analysis provides an efficient and robust method for conducting viral plaque assays. This procedure overview illustrates the three steps carried out on the system. Color brightfield images of the entire well area are captured by the Agilent BioTek Cytation 5. Agilent BioTek Gen5 image analysis tools then identify each plaque from the acquired images, with customized data exports reporting detailed metrics for each sample.

Additionally, by defining the serial dilutions within the protocol, the final viral titer was calculated and reported for each sample.

Conclusion

The viral plaque assay is a well-established method for determining viral titers for lytic viruses. However, conventional analysis methods limit throughput and rely on subjective metrics. Employing automated image capture and analysis protocols on the Agilent BioTek Cytation cell imaging multimode reader and Agilent BioTek Gen5 microplate reader and imager software provides a convenient and robust method for managing viral plaque assays. While this study was conducted on a Cytation 5 using the color brightfield imaging mode, similar protocols have been developed that utilize the inverted camera and color brightfield imaging available on other Agilent BioTek Cytation models and the Agilent BioTek Lionheart automated microscope, as well as the upright color camera available on the Agilent BioTek Cytation 7 cell imaging multimode reader.

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