Viral titration by the Plaque assay

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Abstract

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Protocol

Step 1.

Seed Vero lineage cells (kidney epithelial cells from African green monkey) in 24- well plates at a density of 2 x 10^5 cells/ well.

Step 2.

Maintain in RPMI 1640 medium (Cultilab) supplemented with 10% fetal bovine serum (Cultilab), 2 mM L-glutamine (Gibco) and 100U/50µg penicillin/streptomycin (Sigma-Aldrich) for 24 h at 37°C in a 5% CO2 incubator.

Step 3.

Prepare serial ten-fold dilutions of viral suspension in sterile dilution tubes (10^{-1} up to 10^{-10}).

Step 4.

Label 24-well plate containing confluent Vero cell monolayers for inoculation.

Step 5.

Add 100 µl of the viral suspension without dilution into each of the two wells of 24-well plate.

Step 6.

Add 100 µl of each serial dilution into each of the two wells of 24-well plate and maintain two wells without infection.

Step 7.

Incubate the inoculated plate at 37°C for 1 hour in a 5% CO2 incubator to allow for virus adsorption.

Step 8.

Add 1 ml of 3% semi-solid carboxymethyl cellulose (CMC) overlay medium to each well to limit the virus diffusion.

Step 9.

Maintain the plate for seven days at 37°C in a 5% CO2 incubator.

Step 10.

Incubate cell monolayer with 1 ml of the 10% formol for a minimum 18 hours at room temperature for fixation.

Step 11.

Remove the overlay medium and fixer.

Step 12.

Wash cell monolayer in running water (3 times).

Step 13.

Stain cell monolayer with 1 ml of the 0.04% Violet crystal for 1 hour at room temperature.

Step 14.

Count viral plaques for calculate plaque-forming unit (PFU).