Reovirus Plaque Assay

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Abstract

Assay to obtain plaque forming units of mammalian orthoreovirus

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Protocol

Step 1.

Cells: seed 6-well plates with 1×106 L929 cells per well in a volume of 2 ml complete MEM (Joklik S-MEM supplemented with 5% FBS, 2 mM L-Glutamine, 100 U penicillin per ml, 100 ug streptomycin per ml, 0.25 mg per ml amphotericin B. Incubate at 37°C and 5% CO2 overnight. Alternatively, seed plates with 2×106 cells per well if they are to be used the same day.

Step 2.

Virus dilutions: make serial dilutions of virus samples to be tittered by adding 110 ul of sample to 1 ml of PBS (10-1) into a titer tube or deep-well 96 well plate. Mix by pipetting up and down 16 times and transfer 110 ul to 1ml of PBS for the next dilution (10-2). Continue this process, changing tips between dilutions. Be sure to mix the last sample.

Step 3.

Infection: on the day of infection, label the plates and remove the medium off the cells. Add 110 ul of each virus dilution to each of 2 wells. Incubate at room for 60 min temperature rocking every 10. Melt 2% bacto agar (autoclaved Difco Bacto-agar in water) in microwave and place in 65°C water bath. Warm complete 2X Medium 199 (2X199; supplemented with 5% FBS, 4 mM L-Glutamine, 200 U penicillin per ml, 200 ug streptomycin per ml, 0.5 mg per ml amphotericin B) to 37°C.

Step 4.

Overlay: prepare 2X199/agar mixture by combining equal amounts (1:1 volume:volume) of complete 2X199 and agar. Mix by swirling and allow media to cool slightly. Carefully overlay each well with 3 ml. Incubate at 37°C and 5% CO2 for 2 days.

Step 5.

Feed: on day 2 after infection, prepare 2X199/agar mixture and overlay each well with 2 ml. Incubate at 37°C and 5% CO2 for 4 days.

Step 6.

Stain: on day 6 after infection, prepare 2X199 mixture (using 2x199 media supplemented only with penicillin, streptomycin, and amphotericin B mixed with 2% bacto agat) and add 3 ml of 1% neutral red solution (5 g neutral red in 500 ml water passed through a 0.45 um filter) for each 100 ml. Allow mixture to cool slightly and overlay each well with 2 ml. Incubate at 37°C and 5% CO2 overnight.

Step 7.
Read: count plaques (30-300 plaques per well is an acceptable range) on a light box on day 7 after infection. Calculate plaque titer per ml (PFU/ml).

Warnings

Careful with boiling agar, use hand protection.