Chlorella Virus Plaque Assay Version 2

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Abstract

Adapted from: Van Etten, J. (n.d.). Titering of *Chlorella* Viruses. Retrieved from http://ncv.unl.edu/vanettenlab/
Contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Samantha Coy (srose16@vols.utk.edu) for additional information regarding this protocol.

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Protocol

MBBM Soft Agar

Step 1.

Melt MBBM soft agar and dispense in 3.0 mL aliquots and hold at 45ºC - 50ºC in a water bath

Concentrate Chlorella

Step 2.

Concentrate Chlorella to $4.0*10^8$ cells/mL at 5,000 rpm for 5 min at 4ºC and resuspend with fresh MBBM

*Concentrate so that 0.3 mL can be used per plate

Dilute Chlorella Virus

Step 3.

Dilute virus with 50 mM Tris-HCl, pH 7.8 in 1/10 serial dilutions

*Fresh lysate contains approximately 10^6-10^7 PFU/mL (check the titer prior to plaque assay)

*Dilute sample to have 20-200 plaque forming units (PFU) per plate

Titering

Step 4.

To each 3.0 mL soft agar aliquot, add 0.1 mL Chlorella virus and 0.3 mL Chlorella

*Mix by rolling between palms and pour onto MBBM plate

*Tilt the plate gently to allow the entire surface to be covered

*Allow plate to solidify

Incubation

Step 5.

Incubate at 25ºC in continuous light for 3-4 days or until plaques are visible to count

*Use a sharpie to count plaques once there is a visible contrast