Most Probable Number (MPN) Counts of Cyanophages

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Abstract

Purpose: to measure the infective titer (number of infective elements per mL) of from 1-5 viral samples in a quick and efficient manner.

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Guidelines

(Modified from R. Fu, K. Frois-Moniz & M. Sullivan protocol)

Materials: (assuming 2 replicates per viral sample)

- Two sterile, flat-bottom, 96-well plates for up to 5 viral samples with a capacity for >300 µL
- 30 mL Pro99 or SN medium for each dilution plate plus 30 mL for each sample test plate
- 7 mL late-exponential phase host cells (~3x10^7/mL)
- 60 µL each virus sample
- Multi-channel pipettor with 12x300 µL and 8x1500 µL heads and a 20-100 µL pipette
- Sterile tips for pipettors
- Sterile plastic troughs for cells and growth medium for use with multi-channel pipettors
- Parafilm for sealing lids to prevent evaporation

Labeling example for step 1

		Virus Sample A		*Virus Sample B*		*Virus Sample C*		*Virus Sample D*		*Virus Sample E*		
	1	2	3	4	5	6	7	8	9	10	11	12
-2	A											
-3	B											
-4	C											
-5	D											
-6	E											
-7	F											
0	G											
-1	H											

Calculations: for calculation purposes, each well contains 30 µL of cells mixed with 20 µL phage, diluted with 200 µL medium for a total of 250 µL.

Note: once you have prepared the phage dilutions, the rest of the procedure should be carried out as quickly as possible to minimize (a) possible viral adhesion to the plates and (b) the amount of time that the host cells spend out of the incubator.

Protocol

Labeling

Step 1.

Label the dilution preparation plate using example in guidelines

NOTES

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The numbers “-1” through “-7” represent dilution levels, i.e. 10^{-1} through 10^{-7}-fold dilutions; “0” indicates no phage.

Labeling

Step 2.

Label the sample plates with the date, host, phage name, and any other applicable information (e.g. the light level)

NOTES

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The numbering of the rows and columns must be the same as the dilution plate.

Preparing dilutions in dilution plate

Step 3.

Using a 8x1500 µL multi-channel pipettor, put 270 µL of Pro99 or SN medium in all wells of dilution plate

NOTES

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Will require ~30 mL of medium in a sterile plastic trough.

Preparing dilutions in dilution plate

Step 4.

Using a 100 µL pipettor, dispense 30 µL of virus sample into row H of the dilution plate

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This will be the 10^{-1} dilution of virus.

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Each virus sample is designated two wells per row.

Preparing dilutions in dilution plate

Step 5.

Repeat this for each of the virus samples

Preparing dilutions in dilution plate

Step 6.

Using a 12x300 µL multi-channel pipettor set at 30 µL with 12 tips attached, pipette row H up and down to mix the dilution

Preparing dilutions in dilution plate

Step 7.

Replace the tips and pipette 30 µL from row H into row A (the 10^{-2} row)

Preparing dilutions in dilution plate

Step 8.

Pipette up and down several times to mix

Preparing dilutions in dilution plate

Step 9.

Replace the tips and transfer 30 µL into row B (10^{-3} row)

Preparing dilutions in dilution plate

Step 10.

Repeat this mixing, replacing tips and transferring 4 more times until you are at row F (10^{-7} row)

NOTES

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TIP: using the lid to cover the rows that have not yet had virus put in them helps to prevent cross-contamination of the rows.

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Do not transfer anything to row G since this is the ”no phage” row.

Inoculating cells and phage into the sample test plates

Step 11.

Using a 12x300 µL multi-channel pipettor set to 30 µL with 8 tips attached, pipet 30 µL host cells into all appropriate wells of the sample plate

NOTES

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These are all the wells in columns 2 through 11 (the first and last column of each plate contain medium that provides moisture so that the test wells do not evaporate during incubation).

Inoculating cells and phage into the sample test plates

Step 12.

Reset the pipettor to dispense 20 µL, put on 8 fresh tips and transfer 20 µL from the virus dilution plate to the same corresponding column in the sample test plate

NOTES

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You should replace the tips with fresh ones for each column. If you are testing several different hosts, you can set the pipettor to step up to 10 times at 20 µL and inoculate up to 10 different hosts, each in a separate test plate.

Step 13.

Put the lids on the sample test plates and place in incubator under appropriate light and temperature conditions for each host, and let the virus adsorb to the host for 1 hr

DURATION

01:00:00

Step 14.

Using the 8x1500 µL multi-channel pipettor set to step 6x200 µL, add 200 µL of Pro99 or SN medium to all wells in all sample plates

NOTES

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This will come to 250 µL per well and requires 20 mL per sample test plate.

Step 15.

Seal each plate with parafilm and return plates to the incubator

NOTES

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Plates may be read in 1-2 weeks, however, it might be preferable to get an initial reading on the plates and monitor progress every 2-3 days. We use the Appliskan plate reader, set to read fluorescent emission using a 485 µm excitation filter and a 675 µm (for green fluorescence) or 590 µm (for red fluorescence) emission filter.