PBCV-1 Virus Plaque Assay

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Abstract

PBCV-1 is a large plaque virus (approximately 2-3 mm in diameter). The plaque size of the viruses reflects the length of the virus replication cycle. PBCV-1 has a life cycle of about 6-8 hours. When titering you should use plates that were poured no more than 1 day before the time of use. Variable plaque formation will result if fresh plates are not used. (This is similar to a phage phenomenon.) Pyrex petri dishes do give more uniform plaque formation versus the plastic, however this is not so critical as with slower growing NC64A viruses.

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Guidelines

MATERIALS:

1) MBBM plates with MBBM + 1.5% agar
2) MBBM soft agar with MBBM + 0.75% agar
3) 50 mM Tris-HCl, pH 7.8, sterile, for dilution blanks
4) 13 x 100 mm pyrex tubes, capped and sterile
5) 50°C water bath
6) NC64A culture concentrated to 4.0 X 10^8 cells/ml

Protocol

Pour MBBM plates

Step 1.
Pour the plates either the day of titering or the day before.

NOTES

Irina Agarkova 29 Mar 2016
Fresh plates give more uniform plaque size; old plates (3 days or more) give more erratic results.

Pour MBBM plates

Step 2.
Melt the MBBM soft agar and hold in the 50°C water bath.

NOTES
Irina Agarkova 26 Apr 2016
Melt enough for use. The soft agar can be remelted and used several times.

Pour MBBM plates

Step 3.
Dispense 2.5 ml of soft agar to sterile 13 x 100 mm tubes and hold at 50°C.

NOTES
Irina Agarkova 26 Apr 2016

The soft agar can be made up in quantity in advance and sterilized in 200-250 ml aliquots and stored until ready to use.

Pour MBBM plates

Step 4.
Concentrate the NC64A chlorella to 4.0 X 10^8 cells/ml in the Sorvall centrifuge at 5,000 rpm, 5 min, 4°C and resuspend the pellets with fresh medium.

DURATION
00:05:00

Pour MBBM plates

Step 5.
Concentrate enough so that 0.3 ml can be used for each plate.

Pour MBBM plates

Step 6.
Make up dilution blanks for the virus in the sterile 13 x 100 mm tubes with 50 mM Tris-HCl, pH 7.8.

Pour MBBM plates

Step 7.
Dilute the virus in 1/10 serial dilutions.

Pour MBBM plates

Step 8.
Label the plates.

Titering

Step 9.
Remove 13 x 100 mm tubes of soft agar from the water bath to a test tube rack as needed.

Titering

Step 10.
To each tube, add 0.3 ml of the concentrated chlorella and 0.1 ml of the diluted virus (when titering the NY-2A virus, use 0.05 to 0.1 ml of the concentrated chlorella instead of 0.3 ml).

Titering

Step 11.
Mix briefly (by rolling the tubes between the palms of the hands) and pour the contents of the tube onto the plate.

Titering

Step 12.

Tilt the plate gently until the entire surface of the plate is covered with soft agar.

NOTES

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This needs to be done quickly, as the soft agar will solidify quickly once it has been poured onto the plate.

Titering

Step 13.

Allow the top agar to solidify and stick to the bottom agar (maybe 20 minutes).

DURATION

00:20:00

Titering

Step 14.

Invert the plates with the lid down.

NOTES

Irina Agarkova 26 Apr 2016

So moisture condensation stays in the lid and off the agar surface.

Titering

Step 15.

Incubate the plates at 25°C in continuous light.

Titering

Step 16.

Stack the plates only two deep, as the plaque size will increase if the plates are stacked deeper.

Titering

Step 17.

Plaques will be ready to count after 3-4 days incubation.