Plaque Assay for the Aureococcus anophagefferens Virus (AaV)

Eric Gann

Abstract

We have developed a plaque assay for AaV with a modified protocol from Schroeder et. al 2002.

Protocol modified from:

Schroeder, D.C., et al., *Coccolithovirus* (*Phycodnaviridae*): Characterisation of a new large dsDNA algal virus that infects *Emiliania huxleyi*. Archives of Virology, 2002.147(9): p. 1685-1698.

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Protocol

Preparation

Step 1.

Make $ASP_{12}A$ and autoclave. Do not add solutions after autoclaving.

PROTOCOL

. ASP12A Recipe for culturing Aureococcus anophagefferens

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Step 1.1.

Prepare Trace Metal Solutions I - III, Fe-EDTA Sodium Salt Solution, and Vitamin Solution. Add constituents to 750 mL MilliQ water, shake to dissolve. Add MilliQ water to 1.0 L. Filter sterilize. Trace Metal Solutions and Fe-EDTA Sodium Salt Solution are stored at 4ºC in 15 - 50 mL aliquots, while the Vitamin solution is stored at -20ºC in 5 - 10 mL aliquots.

Step 1.2.

Add anhydrous salts, hydrous salts, macronutrients and Tris Base to 75% desired MilliQ water while stirring to dissolve. Add MilliQ water to final volume, autoclave.

Step 1.3.

Once cooled, add in 1mL of the Trace Metal Solutions, Fe-EDTA Sodium Salt Solution, and Vitamin Solution per 1L of media made.

Bottom Agar

Step 2.

For bottom agar, add low melting agarose to ASP_{12} A for a final concentration of 1%. Sterilize by microwaving. Bring to a boil and let solidify. Repeat three times.

Bottom Agar

Step 3.

After third microwave sterilization, add vitamin solution and trace metal solutions after allowing to cool for 5 minutes. Pour (15 mL) into each Petri dish.

Top Agar

Step 4.

For top agar, add low melting agarose to ASP_{12}A for a final concentration of 0.4%, sterilize by microwaving, then add vitamin and trace metal solutions. Keep at 37º C if performing assay, otherwise let solidify.

NOTES

Ashley Humphrey 23 Jan 2017

Follow microwave sterilization process as listed above for bottom agar.

Protocol for 25 Plaque Assays

Step 5.

Concentrate 1.0 L, week old *Aureococcus* by centrifugation (3400 x g for 2 minutes) at 20ºC.

DURATION

00:02:00

Protocol for 25 Plaque Assays

Step 6.

Resuspend to a total volume of (30 mL) in fresh ASP_{12}A.

Protocol for 25 Plaque Assays

Step 7.

Dilute virus (AaV) in fresh $ASP_{12}A$.

NOTES

Ashley Humphrey 26 Jan 2017

We use this approach to titre lysates, but it should work with other samples where a virus is suspected.

Protocol for 25 Plaque Assays

Step 8.

Combine (0.9 mL) concentrated *Aureococcus* with (0.1 mL) diluted virus or control in 15 mL conical tube, invert to mix, and incubate with cap loosened at 19°C for 30 minutes.

Protocol for 25 Plaque Assays

Step 9.

Add 3 mL of 37°C top agar to 15 mL conical tube, invert to mix and pour on bottom agar.

Protocol for 25 Plaque Assays

Step 10.

Once in plate, gently agitate top agar to ensure even coverage.

Protocol for 25 Plaque Assays

Step 11.

Once the plate is solidified, move to a lighted incubator with appropriate light and temperature.

Protocol for 25 Plaque Assays

Step 12.

Allow to incubate at 19°C with a 14:10 light dark cycle (100 $\mu E/m^{-2} s^{-2}$) for 11 days, face up, to allow plaques to develop.