Pseudomonas aeruginosa bacteriophage PP7

15692-B4™

Description

Pseudomonas aeruginosa bacteriophage PP7 is a virus that was isolated from sewage.
Strain designation: PP7 [ATCC 15692-B2, ATCC 25247-B1]
Deposited As: PP7

Storage Conditions

Product format: Freeze-dried
Storage conditions: 2°C to 8°C

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host: *Pseudomonas aeruginosa* 1C [ATCC 17503, ATCC 25247, ATCC 25375, CIP 104116, PRS 101, Stanier 131] (ATCC 15692)
Medium:
ATCC Medium 129: Nutrient agar/stabs/broth with 0.5% NaCl
Temperature: 37°C
Atmosphere: Aerobic

Handling Procedures

1. Follow general procedures given below for phage propagation.
2. Use *Pseudomonas aeruginosa* strain 1C (ATCC®15692™) as host.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage from freeze-dried or thawed frozen vial:

1. Prepare an actively growing broth culture of the recommended host strain 18-24 hours before opening the phage specimen. The host should be in early log phase.
2. Melt several tubes of soft agar (0.5% agar) and maintain at 45-55°C until ready to use.
3. Prewarm plates of the recommended medium in an incubator for approximately 15-30 minutes prior to the plating of the bacteriophage.
4. Add 50 µL of the host culture to a pre-melted 0.5% agar tube and pipette gently to evenly mix, avoiding the creation of bubbles.
    a. Overlay the agar plate surface with 2.5 - 3 mL of melted 0.5% agar containing the host. Allow overlay to harden.
5. Perform the broth serial dilution in quadruplicate using a 96-well plate.
    a. Dispense 90 µL of broth medium into each well.
    b. Add 10 µL of phage lysate to each of the four 10^{-1} wells. Mix thoroughly by pipetting up and down at least 15 times.
    c. Transfer 10 µL from the 10^{-1} wells to the 10^{-2} wells and mix. Continue the serial dilution until at least 10^{-8}.
    d. Using a multichannel pipette, spot 2 µL of each dilution on the agar overlay. Allow to dry before moving the plates to the appropriate incubator.
6. Incubate plates agar side down at the appropriate temperature. Incubation times will vary depending on the phage. Most only need 12-18 hours. Incubating for too long can make the plaques very difficult to read.

To propagate phage:

1. Open the host organism according to the information on the product sheet.
2. Pick one colony from the isolation plate and homogenize in 5 mL of the appropriate broth. Note: It may be necessary to inoculate a larger volume or more test tubes based on the volume needed for the next amplification.
3. Incubate at the appropriate temperature in a shaking incubator until it reaches OD_{600} of 0.1 – 0.3.
4. Thaw or rehydrate the bacteriophage vial. Use 0.5 mL of the appropriate broth to rehydrate freeze-dried material.

a. Infect each 5 mL culture with 100 µL of the bacteriophage and shake at the appropriate temperature overnight. Prepare a fresh subculture of the host.
b. Centrifuge phage culture at 4000 x g for 10 minutes.
c. Filter the lysate with a 0.2 µm PES sterile filter and store the filtrate at 4°C.
d. Perform a spot titer as per the instructions above.
e. They may also be frozen with or without cryoprotectant. If available, liquid nitrogen storage is the best method for long term storage. Most phage can also be freeze-dried. We use double-strength skim milk mixed half-and-half with the filtrate.
NOTE: Broth propagation methods may also be employed with most phage.

Notes

The propagation host (ATCC 15692) is an opportunistic pathogen therefore it is recommended to incubate titer plates between 7-8 hours to avoid host overgrowing. Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pseudomonas aeruginosa* bacteriophage PP7 (ATCC 15692-B4)

References

References and other information relating to this material are available at www.atcc.org.

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