Pseudomonas sp. bacteriophage Ps-G3

49780-B1™

Description

Pseudomonas sp. bacteriophage Ps-G3 was isolated from a salt pond near Lake Chaplin in Saskatchewan. This bacteriophage should be propagated in broth for best results.

Strain designation: Ps-G3

Deposited As: PsG3

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 sp.* G3 (ATCC 49780)
Medium:
ATCC Medium 1822: 1M PPT medium
Temperature: 30°C
Atmosphere: Aerobic

Handling Procedures

1. Carefully follow procedures given below for phage propagation.
2. *Pseudomonas* sp. G3 (ATCC 49780) is the recommended host.

PROCEDURE FOR THE PROPAGATION OF BACTERIOPHAGE 49780-B1.

To recover phage from freeze-dried or frozen vial:

a. Prepare an actively growing culture of the recommended host strain before opening the phage specimen. The host should be 18-24 hours old.
b. Pick one colony from the #1822 plate and homogenize in 5 mL of #1822 broth. Incubate at 30°C while shaking (170-200 rpm) until growth reaches OD_{600} of 0.07 to 0.1.
c. Add approximately 0.5 mL of the recommended broth to a freeze-dried bacteriophage vial. For a frozen vial, thaw completely. Infect each 5 mL culture with 100 µL of the bacteriophage. Shake at 170-180 rpm in 30°C overnight.
d. Sub culture the host and incubate at 30°C overnight.
e. After 16 to 18 hours, centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate two times with a 0.2 µm or 0.45 µm PES sterile filter. The filtrate can be stored at 4°C.
f. Melt the 0.5% #1822 agar completely. The melted soft agar can be stored at 55°C for up to a week before use.
g. To perform a spot titer, warm one or two plates at 37°C. The soft agar should be brought to 43°C to 45°C until ready to pour. It may be advisable to use a water bath. Overlay the surface with 2.5 mL of melted 0.5% #1822 agar containing 100 µL of the overnight host culture. Allow overlay to harden. This usually won't take longer than 15 to 30 minutes.
h. The phage lysate can be serial diluted in a 96 well plate in quadruplicate (if desired). Aliquot 90 µL of #1822 broth medium into each well. Add 10 µL of phage lysate to each well and mix. Pass 10 µL to each of the next set of wells and mix. Continue to the desired number of passages.
i. Spot 2 µL of each dilution on the plate from step g. Up to 8 dilutions can fit on a 90 mm petri dish. Allow spots to dry then incubate inverted plates at 30°C. This temperature is important to prevent the host from over growing. After overnight incubation, lysis should be visible. At the higher dilutions, individual plaques should be countable.
j. To calculate: pfu/mL = average plaque count / [(dilution factor) ($2x10^{-3}$mL)]
NOTE: Spotting the phage on plates makes visualizing the lysis easier. If phage is added directly to soft-agar before pouring plates, hazy or tiny plaques may be difficult to see. Resistant host bacteria may also mask plaque formation.

To propagate phage:

a. Determine the total volume needed and place this amount of broth in a flask. Add a small amount of overnight host culture to the flask and incubate at 30°C while shaking until the growth reaches OD_{600} of 0.07 to 0.1.

b. Infect with the calculated volume of phage lysate using the following formula. Volume of phage to add (ml) = ($8x10^8$ x total culture volume in ml x OD_{600} x MOI) / phage titer (PFU/ml). Shake at 170-180 rpm at 30°C overnight.
c. Centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate two times with a 0.2 µm or 0.45 µm PES sterile filter. The filtrate can be stored at 4°C.
d. Lysates should remain viable under refrigeration for long periods. 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. ATCC uses double strength skim milk mixed half and half with the filtrate.

Notes

For best results, propagate this bacteriophage in broth.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pseudomonas* sp. bacteriophage Ps-G3 (ATCC 49780-B1)

References

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

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