Clostridium sporogenes bacteriophage 71

17886-B4™

Description

Bacteriophage 71 was isolated from compost. This virus is propagated in *Clostridium sporogenes* strain 213 (ATCC 17886).

Strain designation: 71

Deposited As: 71

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: *Clostridium sporogenes* 213 (ATCC 17886)
Medium:
ATCC Medium 51: AC Broth
ATCC Medium 260: Trypticase soy agar/broth with defibrinated sheep blood
Temperature: 37°C
Atmosphere: Anaerobic

Handling Procedures

1. Follow general procedures given below for phage propagation.
2. Use *Clostridium sporogenes* strain 213 (ATCC 17886) 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 48 hours before opening the phage specimen. Ensure that growth of the host is robust in broth and on agar before starting any bacteriophage workup.
    a. ANAEROBIC CONDITIONS:
        1. Anaerobic conditions for transfer may be obtained by the use of an anaerobic gas chamber.
        2. Anaerobic conditions for incubation may be obtained by any of the following:
            1. Loose screw caps on test tubes in an anaerobic chamber
            2. Loose screw caps on test tubes in an activated anaerobic gas pack jar
            3. Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.
        3. Always use freshly prepared pre-reduced media or pre-reduced media that has been previously prepared but stored under anaerobic conditions.
2. Melt several tubes of #51 soft agar (0.5% agar) and maintain at 55-60°C until ready to use.
3. Prewarm reduced #260 agar plates in an anaerobic incubator for approximately 15-30 minutes prior to the plating of the bacteriophage.
4. Add 300-500 µL of the host culture to a pre-melted 0.5% soft agar tube and pipette gently to evenly mix, avoiding the creation of bubbles.
    a. Overlay the agar plate surface with 3-4 mL of melted 0.5% soft 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 reduced #51 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. Incubate for approximately 23-26 hours. Incubating longer than 26 hours will cause the

plates to dry and the plaques harder to visualize.

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.
    a. 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.

Notes

It's best practice the check the incubating plates often to avoid over drying of agar or host overgrowth.

This phage exhibits best growth without the use of a soft agar overlay.

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.

Broth propagation methods may also be employed with most phage. Unless otherwise noted, ATCC® uses the Adams agar overlay method as described in M. H. Adams' Bacteriophages (Interscience Publishers, Inc., New York, 1959) for routine

phage production.
Medium must be kept anaerobic and inoculated plates incubated in a suitable anaerobic jar or chamber.
Brucella blood agar can be used as an alternative medium.
Store filtrate at 4°C. Storage at 20°C may cause inactivation of the phage.
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: *Clostridium sporogenes* bacteriophage 71 (ATCC 17886-B4)

References

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

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