



Cutibacterium acnes bacteriophage

29399-B1™

Description

The presence of two bacteriophages was confirmed by next generation sequencing. One produces a clear plaque while the other produces a hazy plaque when ATCC 29399, *Cutibacterium acnes* Strain 3, is infected.

Deposited As: *Propionibacterium acnes* phage

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 2

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: *Cutibacterium acnes* 3 (ATCC 29399)

Medium:

ATCC Medium 1490: Modified chopped meat medium

Temperature: 37°C

Atmosphere: Anaerobic

Handling Procedures

1. Follow general procedures given below for phage propagation.
2. Use *Cutibacterium acnes* strain 3 (ATCC® 29399™) 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 24-

72 hours before opening the phage specimen.

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 ATCC Medium #1490 soft agar (0.5% agar) and maintain at 45-55°C until ready to use.
3. Prewarm plates of the recommended medium in an anaerobic incubator for approximately 15-30 minutes prior to the plating of the bacteriophage.
4. Add 300 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 2.5 -3 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 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. 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.
 - 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 OD₆₀₀ 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

ATCC Medium #260 can be used as an alternative agar plate.

ATCC 29399-B1 produces countable plaques within 29-30 hours.

All operations should be carried out under anaerobic conditions or with rapidity placing plates in an anaerobe jar or incubator. Complete lysis occurs in approximately 48 hours.

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: *Cutibacterium acnes* bacteriophage (ATCC 29399-B1)

References

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

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