Staphylococcus aureus subsp. *aureus* bacteriophage 3C

27703-B1™

Description

Staphylococcus aureus subsp. *aureus* bacteriophage 3C is propagated in *Staphylococcus aureus* subsp. *aureus* strain CDC 3C (ATCC 27703). Growth of this bacteriophage requires that certain media are used at specific times; the handling procedure must be followed as written and media cannot be interchanged or substituted.

Strain designation: 3C
Deposited As: 3C

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: *Staphylococcus aureus subsp. aureus* CDC 3C (ATCC 27703)
Medium:
ATCC Medium 260: Trypticase soy agar/broth with defibrinated sheep blood
ATCC Medium 3: Nutrient agar or nutrient broth
ATCC Medium 1831: Nutrient Broth/Agar w/ 400ug/ml Calcium Chloride
Temperature: 37°C; 30°C for titer plates
Atmosphere: Aerobic

Handling Procedures

1. Carefully follow procedures given below for phage propagation.
2. *Staphylococcus aureus* subsp. *aureus* CDC 3C (ATCC 27703) is the recommended host.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

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. Subculture the host on #260 agar and incubate at 37°C overnight.
c. Pick one colony from the #260 plate and homogenize in 5 mL of #3 broth. Incubate overnight at 37°C.
d. In the morning, make a 1:100 dilution of the host culture in #3 broth. Infect the culture with the entire contents of the bacteriophage vial. Shake at 170 rpm at 37°C for 5 to 6.5 hours. After incubating, centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate with a 0.2 µm PES sterile filter then filter again with another 0.2 µm PES sterile filter. If needed, the filtrate can be stored at 4°C.
e. Prior to performing the spot titer, subculture host to a #3 broth and incubate overnight at 37°C.
f. Melt the 0.5% #1831 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% #1831 agar containing 50-100 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 #1831 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. 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. After completing steps, a-d above, subculture the host on #260 agar and incubate at 37°C overnight.
b. Pick one colony from the #260 plate and homogenize in 5 mL of #3 broth. Incubate overnight at 37°C.
c. In the morning, determine the total volume needed and place this amount of #3 broth in a vented flask. Make a 1:100 dilution of the host culture in the flask. Add about 10% of the total volume of phage filtrate at 37°C while shaking at 170 rpm for 5 to 6.5 hours.
d. Centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate with a 0.2 µm PES sterile filter then filter again with another 0.2 µm PES sterile filter. The filtrate can be stored at 4°C.
NOTE: Lysates should remain viable under refrigeration for long periods. Storage at -20°C may cause inactivation of the phage. They may be frozen with 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

Follow the instructions as written above. Do not interchange or substitute media. Recovery and propagation require the use of #260, #3, and #1831 media at different steps. The host may be recovered initially using ATCC Medium 18: Trypticase Soy Agar/Broth; however, all subsequent transfers must follow the procedure outlined above.

Most staphylococcal phages will not produce marked clearing of a broth culture, and a somewhat turbid broth may or may not yield high-titer phage.

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: *Staphylococcus aureus* subsp. *aureus* bacteriophage 3C (ATCC 27703-B1)

References

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

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