Clostridium sporogenes bacteriophage F1

8074-B1™

Description

Strain designation: F1
Deposited As: *Clostridium sporogenes* bacteriophage F1

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* 11797 [L.S. McClung 2002] (ATCC 8074)
Medium:
ATCC Medium 2107: Modified Reinforced Clostridial
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* (ATCC 8074) as host.

ANAEROBIC CONDITIONS:

Anaerobic conditions for transfer may be obtained by the use of an anaerobic gas chamber or placement of test tubes under a gassing cannula system connected to anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:

- Loose screw caps on test tubes in an anaerobic chamber
- Loose screw caps on test tubes in an activated anaerobic gas pack jar
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.

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 from a frozen stock before opening the phage specimen. The host should be 24 hours old when infected with phage
2. Add approximately 1.0 mL of the recommended broth to a freeze dried phage vial, 0.5 mL to a liquid cryovial.
3. Prewarm plates of the recommended medium anaerobically in an incubator. Overlay the surface with 2.5 mL of melted 0.5% agar (same medium) which contains one drop of the 24 hour old host. The soft agar should be maintained at 43°C to 45°C till ready to pour. It may be advisable to use a water bath. Allow overlay to harden.
4. The rehydrated phage can be serially diluted by passing 0.25 mL of the phage into a tube containing 2.25 mL of the broth medium. Repeat for as many passages as desired.
5. 100 µL of each dilution is spotted on the surface of the prepared plates. Allow to dry. Three to four dilutions can be placed on each plate. After overnight anaerobic incubation at 37°C, lysis should be visible. At the higher dilutions, individual plaques should be countable.
6. Many strains may also be titrated without a soft agar overlay. Pipette approximately 1.0 mL of the host onto the surface of each plate. After tilting plate to ensure the entire surface is covered, the excess liquid is aspirated off. After the surface dries, the various dilutions of the phage are dropped onto the surface as before.
   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:

1. Phage may be propagated by preparing plates with the soft agar/host overlay as above and covering the surface with approximately 0.5 mL of the concentrated phage. Alternatively, you may add the phage directly to the melted agar/host before pouring over the plates. For larger amounts, large size T-flasks can be prepared with the recommended agar, and approximately 12.0 mL of melted soft agar/host poured over the surface. Phage is then allowed to run over hardened surface. Phage may also be added directly to melted soft agar before pouring as described above.
2. After 24 hours incubation, the soft agar is scraped off the surface of the agar plates. Centrifuge at about 1000 rpm for 25 minutes to sediment the cellular debris and agar. Conserve the supernatant.
3. This supernatant is passed through a .22 µm Millipore filter and the filtrate may be stored at 4-8°C for a brief time. The phage should be frozen with or without cryoprotectant if kept for more than a few days. If available, liquid nitrogen storage is the best method for long term storage. Most phage can also be freeze dried, using double strength skim milk mixed half-and-half with the filtrate.
   NOTE: 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.

Notes

Anaerobe Systems Brucella Blood Plates (AS-111 or AS-141) can also be used for propagation.

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: *Clostridium sporogenes* bacteriophage F1 (ATCC 8074-B1)

References

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

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