Product Sheet

Escherichia coli
bacteriophage Phi X174
(ATCC® 13706-B1™)

Please read this FIRST

Description

Designation: Phi X174
Deposited Name: Phi X174

Propagation

Medium
ATCC® Medium 129: Nutrient agar/stabs/broth with 0.5% NaCl

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure

1. Follow general procedures given below for phage propagation.
2. Escherichia coli strain C (ATCC® 13706™) is recommended as host.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage from freeze-dried or frozen vial:

a. Prepare an actively growing broth culture of the recommended host strain before opening the phage specimen. The host should be 18-24 hours old.
b. Add approximately 0.25 mL of the recommended broth to a freeze-dried phage vial, 0.5 mL to a liquid cryovial.
c. Pre-warm plates of the recommended medium in an incubator. Overlay the surface with 2.5 mL of melted 0.5% agar (same medium) which contains one or two drops of the 18-24 hour host. The soft agar should be maintained at 43°C to 45°C until ready to pour. It may be advisable to use a water bath. Allow overlay to harden.
d. The re-hydrated phage can be serially diluted by passing 0.5 mL of the phage into a tube containing 4.5 mL of the broth medium. Repeat for as many passages as desired.
e. 0.1 mL 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 incubation, lysis should be visible. At the higher dilutions, individual plaques should be countable.
f. 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:

a. Phage may be propagated by preparing plates with the soft-agar/host overlay as above and then 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-tubes 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.
b. 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.
c. This supernatant is passed through a .22 um Millipore filter and the filtrate stored at 4-8°C. 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.

NOTE: Both 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

Resistant bacteria colonies will be seen in areas of complete lysis. Repeated transfer may bring out a "giant" mutant plaque type, so avoid excessive passage. If phage is added directly to overlay before pouring over plate, it will not be easy to observe plaques. The same is true for broth culture. The production of resistant
host bacteria prevents the broth from clearing. This phage does not preserve well in the lyophilized state. It is best stored frozen, ideally at liquid nitrogen temperatures. The vial you receive is a frozen cryovial that contains bacteria-free lysate and glycerol as cryoprotectant. It does not have to be kept frozen, but should be refrigerated until used.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

© ATCC 2018. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [08/17]