Please read this FIRST

**Storage Temp.**
- Frozen: -80°C or colder
- Freeze-Dried: 2°C to 8°C

**Live Culture: See Propagation Section**

**Biosafety Level**
- Level 1

**Intended Use**
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

**Citation of Strain**
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Escherichia coli bacteriophage T4* (ATCC® 11303-B4™)

**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. Use *Escherichia coli* strain B (ATCC® 11303™) as host.

**GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE**

To propagate phage:

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. 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 43-45°C until ready to pour. It may be advisable to use a water bath.

   Allow overlay to harden.

c. Open the vial according to the enclosed instructions. The vial of phage can be serial diluted using 1:10 dilutions in #129 broth.

d. One drop 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.

e. 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 the 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 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. 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 43-45°C until ready to pour. It may be advisable to use a water bath.

   Allow overlay to harden.

c. Open the vial according to the enclosed instructions. The vial of phage can be serial diluted using 1:10 dilutions in #129 broth.

d. One drop 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.

e. 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 the 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.

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

**References**

*Please read this FIRST*

**Intended Use**
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

**Citation of Strain**
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Escherichia coli bacteriophage T4* (ATCC® 11303-B4™)
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.

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