



Product Sheet

Escherichia coli

bacteriophage 250 (ATCC® 11303-B20™)

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
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 250* (ATCC® 11303-B20™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: 250
Deposited Name: 250

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 recover phage from freeze-dried or thawed LN₂ vial:

- a. Prepare an actively growing broth culture of the recommended host strain before opening the phage specimen. The host should be in early log phase.
- 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) that contains one or two drops of the freshly grown host. The soft agar should be maintained 43-45°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 0.25 mL of the phage into a tube containing 2.25 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 24 hours 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 covering the surface with approximately 0.5 mL of the concentrated phage. Or, 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.
- b. After 24 hours incubation, or when lysis is observed, 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 µm 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. We use 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

Plaques are medium and clear with a slight halo.

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



Product Sheet

Escherichia coli *bacteriophage 250* (ATCC® 11303-B20™)

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
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 250* (ATCC® 11303-B20™)

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 2015. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [09/08]

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor