



Escherichia coli bacteriophage 221

11303-B22™

Description

Strain designation: 221

Deposited As: 221

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: *Escherichia coli* B [CIP 103914, NCIB 11595, NRC 745] (ATCC 11303)

Medium:

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

Temperature: 37°C

Atmosphere: Aerobic

Handling Procedures

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.
- Add approximately 0.25 mL of the recommended broth to a freeze-dried phage vial, 0.5 mL to a liquid cryovial.
 - 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.
 - 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.
 - 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.
 - 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:

- 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.
- 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.
- This supernatant is passed through a .22 µm Millipore filter and the filtrate

***Escherichia coli* bacteriophage 221**

11303-B22

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

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: *Escherichia coli* bacteriophage 221 (ATCC 11303-B22)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media

formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with

***Escherichia coli* bacteriophage 221**

11303-B22

or arising out of the customer's use of the product. While reasonable effort is made to ensure 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 material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2022-10-22

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
