ATCC

Product Sheet

Klebsiella pneumoniae subsp. pneumoniae bacteriophage 60 (ATCC® 23356-B1™)

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: *Klebsiella pneumoniae subsp. pneumoniae bacteriophage 60* (ATCC® 23356-B1™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
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800.638.6597 or 703.365.2700
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Or contact your local distributor

Description

Designation: 60
Deposited Name: 60

Propagation

Medium
ATCC® Medium 3: Nutrient agar or nutrient broth

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure

1. Follow general procedures given below for phage propagation.
2. Use *Klebsiella pneumoniae* subsp. *pneumoniae* strain A-1 (ATCC® 23356™) as host.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE
To recover phage from freeze-dried or thawed LN_2 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 1.0 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 to 45°C till 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 0.1 mL of the phage into a tube containing 0.9 mL of the broth medium. Repeat for as many passages as desired.
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 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 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 pinpoint to 0.4 mm and hazy with a slightly irregular margin and no halo. Phage is osmotically sensitive.
Additional information on this culture is available on the ATCC® web site at www.atcc.org.

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References

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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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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.
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