Mycobacterium vaccae bacteriophage B5 (ATCC® 15483-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: Mycobacterium vaccae bacteriophage B5 (ATCC® 15483-B1™)

Description
Designation: B5
Deposited Name: B5

Propagation
Medium
ATCC® Medium 1395: Middlebrook 7H9 broth with ADC enrichment
ATCC® Medium 173: Middlebrook 7H10 agar with Middlebrook OADC enrichment

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure
1. Follow general procedures given below for phage propagation.
2. Use Mycobacterium vaccae strain SN 920 (ATCC® 15483™) 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 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 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 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.

Notes
Use a dense suspension of the host to seed the plates.
Plaques vary in size and are clear with large halos.

References
Additional information on this culture is available on the ATCC® web site at www.atcc.org.
**Biological Safety 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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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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