Escherichia coli bacteriophage Lambda W60

BAA-3382™

Description

This product is an ATCC manufactured and accessioned progeny of 97537.
Strain designation: LambdaW60 [W60]
Deposited As: ATCC accessioned progeny of *Escherichia coli* bacteriophage Lambda W60 cited as 97537.
Shipping information: bacteria-free lysate

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* MG1655 (ATCC 47076)
Medium:
ATCC Medium 1065: LB Agar/Broth, Miller
Temperature: 37°C
Atmosphere: Aerobic

Handling Procedures

1. Follow general procedures given below for phage propagation.
2. *Escherichia coli* strain MG1655 (ATCC 47076) is the recommended host.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage from freeze-dried or frozen vial:

a. Prepare an actively growing culture of the recommended host strain before opening the phage specimen. The host should be 18-24 hours old.
b. Pick one colony from the isolation plate and homogenize in 5 mL of the appropriate broth. Incubate at 37°C while shaking until the growth reaches OD $_{600}$ of 0.3 to 0.4.
c. Infect each 5 mL culture with 300 µL of the bacteriophage. Add 25 µL each of 2M $CaCl_2$ and 2M $MgCl_2$. Shake at 130 rpm at 37°C overnight. After 16 to 18 hours, centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate with a 0.2 µm PES sterile filter then filter again with another 0.2 µm PES sterile filter. The filtrate can be stored at 4°C.
d. To perform a spot titer, warm one or two plates at 37°C. Overlay the surface with 2.5 mL of melted 0.5% agar (same medium) which contains 50 µL of an 18-24 hour host culture. The soft agar should be maintained at 43°C to 45°C until ready to pour. It may be advisable to use a water bath. Allow overlay to harden.
e. The phage lysate can be serial diluted in a 96-well plate in quadruplicate (if desired). Aliquot 90 µL of broth medium (containing 25 µL each of $CaCl_2$ and $MgCl_2$ per 5 mL of broth) into each well. Add 10 µL of phage lysate to each well and mix. Pass 10 µL to each of the next set of wells and mix. Continue to the desired number of passages.
f. Spot 2 µL of each dilution on the plate from step d. Up to 8 dilutions can fit on a 90 mm petri dish. After overnight incubation, lysis should be visible. At the higher dilutions, individual plaques should be countable. pfu/mL = average plaque count / [(dilution factor) ($2x10^{-3}$mL)]
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. Determine the total volume needed and place this amount of broth in a flask. Add a small amount of overnight host culture to the flask and incubate at 37°C while shaking until the growth reaches OD_{600} of 0.3 to 0.4.
b. Infect with the calculated volume of phage lysate using the following formula. Volume of phage to add (ml) = ($8x10^8$ x total culture volume in ml x OD_{600} x

MOI) / phage titer (PFU/ml). Add 25 µL each of 2M $CaCl_2$ and 2M $MgCl_2$ per 5 mL of broth. Shake at 130 rpm at 37°C overnight.

c. Centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate with a 0.2 µm PES sterile filter then filter again with another 0.2 µm PES sterile filter. The filtrate can be stored at 4°C.
NOTE: 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. ATCC® uses double strength skim milk mixed half and half with the filtrate. Most phage can also be freeze-dried. ATCC® uses double strength skim milk mixed half and half with the filtrate.

Notes

The host strain (ATCC 47076) is a very robust culture. Only 50 µL are need per tube of soft agar.
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 Lambda W60 (ATCC BAA-3382)

References

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

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Revision

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