Escherichia coli bacteriophage PR772

BAA-769-B1™

Description

This is a PR4-like bacteriophage. It is sensitive to organic solvents and adsorbs to tips of pili coded for by IncP-1, N, and W plasmids.
Strain designation: PR772 (HER 221)

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder

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* K12 J53-1(R15) [HER 1221] (ATCC BAA-769)
Medium:
ATCC Medium 18: Trypticase Soy Agar/Broth
Temperature: 37°C
Atmosphere: Aerobic

Handling Procedures

1. Carefully follow the procedures given below for phage propagation.
2. *Escherichia coli* strain K12 J53-1(R15) (ATCC BAA-769) is the recommended host for propagation.

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage from freeze-dried or frozen vial

Day 1

a. Prepare an actively growing culture of the recommended host strain before opening the phage specimen. The host should be 18-24 hours old.
    1. In some instances, the host growth requirements will differ from that of the phage. Because the phage culture will only be as robust as the host culture, it is best to choose conditions according to the preference of the host bacterium.
    2. Typically, the host will grow in the same medium as the phage, but there may be some exceptions.
b. Subculture the host on #18 agar and incubate at 37°C overnight.

Day Two

c. Based off experimental design determines the total volume needed before opening of phage.
d. Pick one colony from step b. and homogenize in 5 mL of #18 broth.
e. Incubate at 37°C in a shaking incubator (160-180 rpm) until it reaches OD $_{600}$ of 0.1 – 0.3. Use a spectrophotometer to take measurements following the manufacturer's instructions.
    1. The culture should be checked at 1 hour and every 15 minutes thereafter until the correct measurement is achieved.
f. Thaw or rehydrate the bacteriophage seed vial. Use 0.5 mL of #18 broth to rehydrate if necessary.
g. Infect a 5 mL culture with 100 µL of the bacteriophage. Store any remaining phage at 4°C.
    1. Perform a purity check by inoculating a blood plate and incubating at 37°C. If desired, inoculate a maintenance medium plate to check for host growth.
    2. Inoculate a fresh subculture of the host and incubate statically at 37°C overnight.

Day Three

h. Centrifuge phage culture at 4000 g for 10 minutes. (This step is optional depending on the density of the culture it may make filtering easier). To convert g to rpm, consult the user manual for each centrifuge. There

may be a conversion tool online. 4000 g usually converts to an rpm between 6000-6500 for benchtop models.

i. Filter the lysate (supernatant) with a 0.2 to 0.45 µm PES sterile filter and store the filtrate at 4°C.
j. Prior to performing the spot titer, subculture host to a #18 broth and incubates overnight at 37°C (in case another round of amplification will be necessary the following day).
k. Melt 0.5% agar completely. The melted soft agar can be stored at 55°C for up to a week before use. Then move to the spot titer steps below.

Performing a spot titer

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.

l. To perform a spot titer, warm one or two plates at 37°C. The soft agar should be brought to 43°C to 45°C until ready to pour. It may be advisable to use a water bath.
m. Overlay the surface with 2.5 mL of melted 0.5% agar containing 100 µL of the overnight host culture. Allow overlay to harden. This usually won't take longer than 15 to 30 minutes.
n. The phage lysate can be serial diluted in a 96 well plate in quadruplicate (if desired). Aliquot 90 µL of #18 broth medium into each well.
o. Add 10 µL of phage lysate to each well and mix. Pass 10 µL to each of the next set of wells and mix. Continuing to the desired number of passages.
p. Spot 2 µL of each dilution on the plate from step o. Up to 8 dilutions can fit on a 90 mm petri dish. Incubate plates at 37°C, inverted is not recommended as it can affect the spots that are spotted. Note: If over-incubated, plaques can continue to enlarge. Given sufficient numbers of plaques, this may result in confluent lysis.

Day Four

This could be a stopping point depending on the total volume achieved from the first round of amplification and the total volume needed for experimentation. Count the plaques in each spot of the dilution with the best growth. Calculate the average count. Use the formula below to determine the pfu/mL.

1. pfu/mL = average plaque count / [(dilution factor) ($2x10^{-3}$ mL)]

To propagate phage:

q. 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 0.1 – 0.3.
r. 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). Shake at 160-180 rpm at 37°C overnight.
s. Centrifuge phage culture at 4000 g for 10 minutes. Filter the lysate with a 0.2 µm or 0.45 µm PES
sterile filter. The filtration can be stored at 4°C.
t. 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 phages can also be freeze-dried. ATCC® uses double strength skim milk mixed half and half with the filtrate.
u. Lysates should remain viable under refrigeration for long periods. Storage at 20°C may cause inactivation of the phage. They may be frozen with cryoprotectant. If available, liquid nitrogen storage is the best method for long term storage. Most phages can also be freeze-dried. ATCC® uses 20% Glycerol half and half with the filtrate.

Notes

This is a PR4-like phage, sensitive to organic solvents and adsorbs to tips of pili coded for by IncP-1, N & W plasmids.
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 PR772 (ATCC BAA-769-B1)

References

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

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