Kehishuvirus tikkala

BAA-3231-B1™

Description

Bacteroides cellulosilyticus bacteriophage Kehishuvirus tikkala is a virus that was isolated in 2018 from wastewater in California.
Strain designation: Bc01

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: *Bacteroides cellulosilyticus* WH2 (ATCC BAA-3231)
Medium:
ATCC Medium 2713: Wilkins-Chalgren Anaerobe Medium
Temperature: 37°C
Atmosphere: Anaerobic

Handling Procedures

1. Follow general procedures given below for phage propagation.
2. *Bacteroides cellulosilyticus* (ATCC BAA-3231) is the recommended host.

ANAEROBIC CONDITIONS:

Anaerobic conditions for transfer may be obtained by the use of an anaerobic gas chamber or placement of test tubes under a gassing cannula system connected to

anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:

- Loose screw caps on test tubes in an anaerobic chamber
- Loose screw caps on test tubes in an activated anaerobic gas pack jar
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained

GENERAL PROCEDURES FOR THE PROPAGATION OF BACTERIOPHAGE

To recover phage frozen vial:

1. Before opening the phage vial, pre-reduce all media and prepare an actively growing culture of the recommended host strain. The host should be 16-18 hours old.
2. Add $MgCl_2$ to a fresh, pre-reduced tube of broth to a final concentration of 10 mM. Inoculate with a few drops of host culture. Incubate at 37°C while shaking (160-180 rpm) until the tube is lightly turbid (this may take several hours).
3. Thaw cryovial. Infect each 5 mL culture tube with 100 µL of the bacteriophage. Shake at 160-180 rpm in 37°C overnight. After incubation, 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 filtrate can be stored at 4°C.
4. Prior to performing a spot titer, pre-reduce and warm one or two plates at 37°C. Plates can be #2713 or anaerobic blood plates (plaques may be easier to see against a dark background agar).
5. Melt the soft agar (0.5% agar added to the recommended medium) and add $MgCl_2$ to a final concentration of 10 mM. Maintain at 43°C to 45°C in an anaerobic environment until ready to use. It is best to allow the melted agar to remain at this temperature for about an hour to ensure that it has cooled to 43°C to 45°C. Warmer temperatures may kill the host.
6. Add 0.5 – 1 mL of the host culture from step 1 to each 9 mL (approximately) of soft agar from step 5 above. Immediately overlay the surface of each plate with 4-5 mL. Allow the overlay to harden for 10 to 20 minutes in an anaerobic environment.
7. The phage lysate can be serial diluted in a 96 well plate in quadruplicate (if desired). Aliquot 90 µL of broth medium + 10 mM $MgCl_2$ into each well. Add 10 µL of phage filtrate from step 3 to each well and mix. Transfer 10 µL from each well of the first dilution to each well of the second dilution and mix. Continue

to the desired number of dilutions.

8. Spot 2 µL of each dilution on the plate from step 6. Up to 8 dilutions can fit on a 90 mm petri dish. After 24-48 hours of anaerobic incubation, lysis should be visible. At the higher dilutions, individual plaques should be countable. To calculate pfu/mL, use the following formula: pfu/mL = average plaque count / [(dilution factor) ($2x10^{-3}$ mL)]
9. 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:

1. Determine the total volume needed and place this amount of broth in a flask to pre-reduce in an anaerobic environment. Add $MgCl_2$ to a final concentration of 10 mM. Add a small amount of overnight host culture to the flask and incubate at 37°C while shaking until the broth is lightly turbid (this may take several hours).
2. 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 in an anaerobic environment.
3. 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 filtrate can be stored at 4°C.
4. 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.
5. Note: To achieve the highest PFU and total volume, the broth method detailed above has demonstrated the best results if the process is followed exactly. However, if any of the equipment are not available or if the technique is not possible for other reasons, the Adam's Overlay method described below will provide adequate results for smaller volumes at satisfactory titer count. (Adams agar-overlay method as described in M. H. Adams' Bacteriophages, Interscience Publishers, Inc., New York, 1959)

To recover phage using the Adam's Overlay method:

1. Before opening the phage vial, prepare an actively growing broth culture of

the recommended host strain. The host should be in early log phase. Times vary according to species but usually no more than 16 to 18 hours incubation. Pre-reduce and pre-warm plates of the recommended medium in a 37°C incubator. Plates can be #2713 or anaerobic blood plates (plaques may be easier to see against a dark background agar).

2. Melt the soft agar (0.5% agar added to the recommended medium) and add $MgCl_2$ to a final concentration of 10 mM. Maintain in an anaerobic environment at 43°C to 45°C until ready to use. It is best to allow the melted agar to remain at this temperature for about an hour to ensure that it has cooled to 43°C to 45°C. Warmer temperatures may kill the host.
3. Add 0.5 – 1 mL of the host culture from step 1 to each 9 mL (approximately) of soft agar from step 3 above. Immediately overlay the surface of each plate with 4-5 mL. Allow the overlay to harden for 10 to 20 minutes.
4. Thaw cryovial. Transfer the entire contents of the vial into a test tube of the recommended broth medium + 10 mM $MgCl_2$. Perform a 1:10 serial dilution for as many passages as desired.
5. One drop of each dilution is spotted on the surface of a prepared overlay plate. Allow to dry. Up to four dilutions can be placed on each plate, however, it may be difficult to keep the spots from running into each other. This is up to the discretion of the biologist.
6. Incubate anaerobically at the recommended temperature for 24-48 hours.

To propagate phage:

1. Phage may be propagated by preparing overlay plates as in steps 3 & 4 above and covering the surface with approximately 0.5 mL of the concentrated phage. Alternatively, you may add the phage directly to the melted agar with host before pouring over the plates.
2. For a larger amount, large-sized T-flasks can be prepared with the recommended agar and approximately 12.0 mL of melted soft agar + 10 mM $MgCl_2$ with host poured over the surface. Allow the overlay to harden. Thaw cryovial. Transfer the entire contents of the vial to the T-flask and allowed to run over the hardened surface.
3. Incubate 24-48 hours in an anaerobic environment. When lysis is observed, scrape the soft agar off the surface of the agar plates or T-flask. Centrifuge at about 1000 rpm for 25 minutes to sediment the cellular debris and agar. Draw off the supernatant and keep.
4. The supernatant is passed through a .22 µm Millipore filter. The filtrate is kept

and stored at 4°C to 8°C. A second filtration may be necessary to completely remove the host.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Kehishuvirus tikkala* (ATCC BAA-3231-B1)

References

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

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