Bacteriophage (Coliphage)

Conditions for Customer Ownership

We are a USDA compliant facility and hold all necessary permits to transport our organisms. Each state is assisted by the USDA to determine which organisms can be transported across state lines. Some organisms may require end-user permits. Please contact your local regulatory authorities with questions or concerns. To access permit conditions, click here.

Never purchase living specimens without having a disposition strategy in place. Live specimens should not be released into the wild! Please dispose of any unwanted organisms using the guidelines below.

Bacteriophage vs. Coliphage

Bacteriophages are viruses that attack bacteria. Each phage has a highly specific host and any given phage will act only on its own particular species or group of species of bateria. Phages are commonly named in reference to their host and thus a phage that attacks *Staphylococcus* is called staphylophage, while one that attacks *E. coli* a coliphage.

Primary Hazard Considerations

Coliphages are viruses that attack *Escherichia coli* bacteria. Be careful when working with phages as they can host in your bacterial collection, contaminating and potentially destroying them. To minimize risks of contamination, remove all cultures and equipment that you are not using from the work area. Wipe down the area with alcohol both before and after working with bacteriophages.

Availability

Phages are available year round. 150 µL of phage will arrive in a plastic vial with the titer indicated on the label. The concentration of infective viral particles per milliliter of culture suspension is referred to as the titer.

Phage	Bacterial Host	Characteristic
T2 (T2r+) T3 (T3r+)	*E. coli* B *E. coli* B	Wild type; rapid lysis. Most frequently used in genetic studies. Wild type; rapid lysis
T4 (T4r+)	*E. coli* B	
T4r	*E. coli* C *E. coli* B	Wild type; rapid lysis. Less sensitive to UV radiation than T2. Rapid lysis. Mutant of T4r+. Non-reverting plaque morphology.
T4rIIA	*E. coli* B	Point mutation in cistron A, segment 4. Reversion rate to wild type approximately 10^6.
T4rIIB	*E. coli* B	Point mutation in cistron B; pseudoallele of T4rIIA. Reversion rate to wild type approximately 10^6.
ϕX174	*E. coli* C	Minute polyhedral from, 25 µm in diameter. Circular single-stranded DNA, close relative of S13 phage.
T7	*E. coli* B	Good for plaque morphology and demonstrations of cations on plaque growth.
	E. coli D	Rapid lysis. Produces large plaques.

Care

Bacteriophages can be stored in the refrigerator for up to six months.

Media

Bacteriophages are stored and diluted in tryptic soy broth.

Information

- A typical phage is a particle close in size to that of a large molecule. It consists of a unit of nucleic acid encased in a protein coat. Like all viruses, they rely upon the host to provide the necessary activities and materials for their reproduction. A typical coliphage looks like a tadpole with an expanded head and elongated tail. When attacking, the coliphage uses its tail to attach itself to the bacterium, and then like a syringe and needle it penetrates the cell wall and collapses its head, injecting DNA into the host. The viral nucleic acid directs the metabolism of the bacterial host in the synthesis of new viral DNA and other materials for making a complete virus. After replication, the bacteriophages are released by a sudden rupture of the host cell wall (lysis) and are marked by a sudden decrease in turbidity of the bacterial suspension. The new viral particles are then free to infect other susceptible bacteria. The concentration of viral particles per milliliter of culture suspension is referred to as the titer.
- Successful culture of the T-series phages requires a sensitive strain of bacteria, generally *E. coli* B. Proper sterile technique must be observed when culturing to insure success as well. For optimal growth of the phage, air should be gently bubbled through the medium. Growth can be achieved without bubbling by shaking the flasks at intervals; however, a lower titer will develop.

Determination of Titer

- A simple method of determining coliphage titer involves serial dilution of the coliphage stock followed by the double agar technique for plaque counts. The bacteria grow as tiny subsurface colonies forming a translucent layer over the agar plate, while the plaques appear as clear patches in the translucent layer of bacterial growth.

  Materials Needed

  - 6 Tryptic soy agar Petri dishes (20 mL each)
  - 6 Soft tryptic soy agar tubes (2 mL each)
  - 11 Tryptic soy broth tubes (9 mL each)
  - Agar slant culture of *E. coli* B

Procedure

1. Label the TSA plates 10^{-6}–10^{-10}, leaving one dish labeled as "control". Allow the plates to warm to room temperature to ensure a uniform pour of the soft agar.
2. Label the agar tubes 10^{-6}–10^{-10}, leaving one tube labeled as "control".
3. Label the broth tubes 10^{-1}–10^{-10}, leaving one tube labeled as "control". These will be used for a serial dilution.
4. Place the agar tubes into a beaker of boiling water and heat until the agar melts. Then transfer the tubes to a 46–50 °C water bath until needed.
5. Using aseptic technique, pipet 1 mL of the phage stock into dilution tube 10^{-1}, mix thoroughly and transfer 1 mL from tube 10^{-1} to 10^{-2}. Repeat until you reach 10^{-10}, using a different sterile pipet for each dilution. Don't add any phage to the control tube.
6. Prepare a broth suspension of *E. coli* B by adding 5 mL of tryptic soy broth to the slant culture and washing off the bacteria by gently shaking the tube. Pipet two drops of the suspension into each soft agar tube using a 1 mL pipet.

7. Pipet 1 mL of the blank tryptic soy broth to the blank soft agar tube containing *E. coli* B and pour it onto the corresponding Petri dish. Gently swirl the closed dish with a circular motion to form a uniform layer of soft agar. This will serve as your control and should have no plaques after incubation.
8. Using your dilution series tubes 10^{-6}–10^{-10}, remove 1 mL of phage stock and transfer it to the corresponding soft agar tube containing *E. coli* B. After mixing, immediately pour it onto the corresponding plate so the soft agar doesn't harden in the tube. Gently swirl the closed dish to distribute a uniform layer of agar.
9. After the plates have set, place them in an inverted position in the 37°C incubator for 18–24 hours. The titer can then be determined.

Discussion and Interpretation of Results

Theoretically, because of the even distribution of *E. coli* B and coliphage across the plate, a single coliphage will adsorb onto a single bacterium and lyse the bacterium as it reproduces. The liberated coliphage particles then infect surrounding host cells and repeat the cycle. Eventually this produces a clear area, or plaque, in the layer of bacteria. By picking a plate that has between 30 and 300 colonies, an accurate statistical estimate of phage titer can be made.

Example

- The plate made from serial dilution tube 10^{-7} has 138 plaques. The titer, or number of infective particles/mL of stock suspension, is then equal to $138/10^{-7}$ or 138×10^{7} or 1.38×10^{9}.
- A possible error in the titer can occur when more than one phage adsorbs to a bacterium or when two phages adsorb onto bacteria that are very close and give rise to one plaque when there should be two. This is the reason that it is essential to pick a plate of 30 to 300 colonies. The control plate should be used for comparison to avoid confusion.

Disposition

When finished with your bacteriophage, please dispose of it in one of the following ways:

- Use a 20% bleach solution for 10 minutes (ensure the culture does not open until the culture is submerged in solution in order to ensure no releasing of the organism into the environment).
- Place the organism in 70% isopropyl alcohol for 24 hours (ensure the culture does not open until the culture is submerged in solution in order to ensure no releasing of the organism into the environment).
- Autoclave the organism @ 121°C for 15 minutes in an autoclavable bag. The Petri dish it is contained in will melt in an autoclave, so be sure to bag your organism and close securely before autoclaving.

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