HiPer® Phage Titration Teaching Kit

Product Code: HTM003

Number of experiments that can be performed: 10

Duration of Experiment

Protocol: 4 days
Day 1: Preparation of media and revival of host
Day 2: Inoculation of host
Day 3: Phage titration
Day 4: Observation and calculation

Storage Instructions

- The kit is stable for 6 months from the date of manufacture
- Store Phage Lysate, *E. coli* Host and 20% Maltose solution (Sterile) at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To study the virulent property of lambda phage on specific host and to determine the phage titre by plaque assay method.

Introduction:

Viruses, which infect bacteria, are known as bacteriophages. The activity of the bacteriophage was first described by Frederick Twort in 1915. Lambda phage was discovered by Esther Lederberg in 1950. Bacteriophages which can be used as vectors are called as phagemids.

Bacteriophages contain nucleic acid and protein. The nucleic acid can be either DNA or RNA but not both and it can exist in various forms. The nucleic acid of phages often contains unusual or modified bases. These modified bases protect phage nucleic acid from nucleases that break down host nucleic acid during phage infection. Most phages range in size from 24-200 nm in length. T4 is among the largest phage which is approximately 200 nm long and 80-100 nm wide.

The basic structural features of bacteriophages are:

1. Head or Capsid - All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) and others are filamentous. The head or capsid is composed of nucleic acid and many copies of one or more different proteins. The head acts as the protective covering for the nucleic acid.

2. Tail - The tail is a hollow tube through which the nucleic acid is passed during infection. The size of the tail can vary. Some phages do not have a tail structure. In the more complex phages like T4, the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail, the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances, other structures are involved in binding of the phage particle to the bacterium.

Principle:

Bacteriophage lambda is a temperate phage which can exist in two cycles: the lytic cycle which involves infection, replication and lysis with the release of progeny phage, and the lysogenic cycle in which the phage chromosome integrates within the host chromosome. The virus identifies specific receptors e.g. maltose binding protein on the host cell surface. To facilitate the adsorption of the phage particle, the host is grown in a medium containing maltose and magnesium. When the host cell is infected with a single phage and lysed, it releases progeny phage which can diffuse to neighboring cells and infect them and as a result a circular area of cell lysis appears on a turbid lawn of cells. These clear zones formed on a lawn of cells are called plaques. The titre of the bacteriophage can be determined by doing the plaque assay. Phage titration assay includes the appropriate dilution of the phage with the host and plating the dilution on a suitable medium.

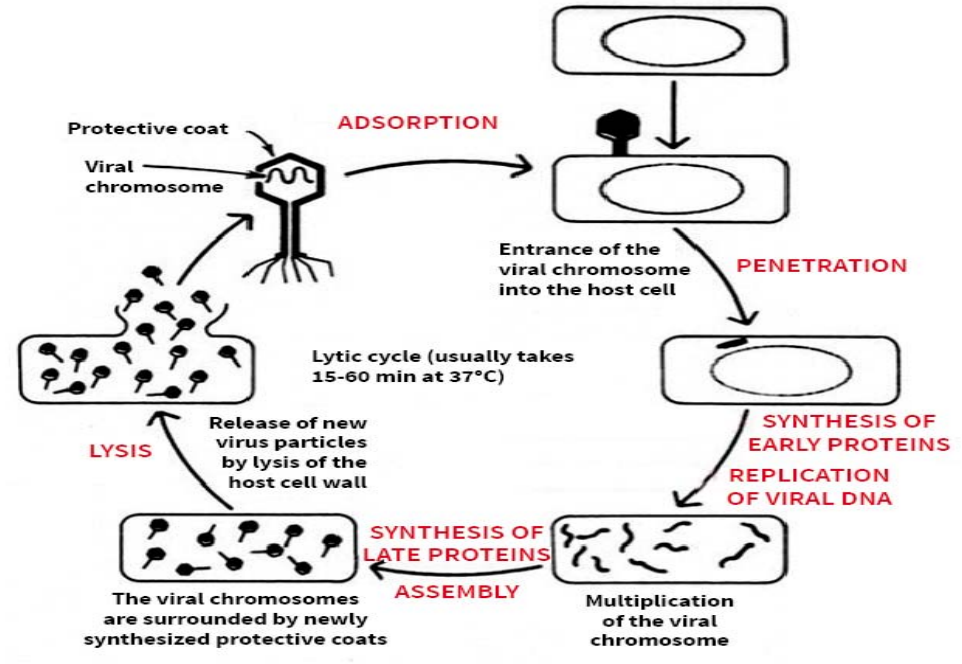


Fig1: Lytic Cycle of Bacteriophage

Kit Contents:

This kit can be used to determine the phage titre by plaque assay method.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	TKC027	*E.coli* Host	1 No.	2-8°C
2	TKC058	Phage lysate	0.12 ml	2-8°C
3	M1245	Luria Bertani (LB) Broth	38 g	RT
4	MB053	Agar Powder, Bacteriological	27 g	RT
5	RM014	Tryptone	0.7 g	RT
6	MB023	Sodium chloride (NaCl)	3.5 g	RT
7	TKC102	SM buffer (Sterile)	75 ml	RT
8	TKC103	20 % Maltose Solution (Sterile)	14 ml	2-8°C
9	TKC060	20 % Magnesium chloride Solution (Sterile)	6.5 ml	RT
10	PW1139	Collection Tubes, Polypropylene (2.0 ml)	70 Nos.	RT

Materials Required But Not Provided:

Glasswares: Sterile Test tubes, Petri dishes, Conical flask, Measuring cylinder
Other requirements: Incubator, Spectrophotometer, Microwave/ hotplate/burner, Micropipettes, Tips, Sterile Distilled water

Storage:

HiPer® Phage Titration Teaching Kit is stable for 6 months from the date of manufacture. On receipt, Phage lysate, 20% Maltose solution (Sterile) and *E. coli* host should be stored at 2-8°C. Other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. **Preparation of LB (Luria Bertani) agar plates (150 ml):** Dissolve 3.75 g of LB media and 2.25 g of agar in 150 ml of sterile distilled water. Sterilize by autoclaving and pour on sterile petri plates.
3. **Preparation of TBM for one experiment (Tryptone Broth with Maltose, 25 ml):** Add 0.025 g of Tryptone, 0.125 g of NaCl and make up the volume to 25 ml with sterile distilled water and autoclave. Add 0.25 ml of 20% $MgCl_2$ and 0.5 ml of 20% Maltose solution before use.
4. **Preparation of soft agar for one experiment (30 ml):** Add 0.15 g of agar, 0.03 g of Tryptone, 0.15 g of NaCl and make up the volume to 30 ml with sterile distilled water. Melt the agar; dispense it into tubes (not provided) and autoclave. Add 0.3 ml of 20% $MgCl_2$ and 0.6 ml of 20% Maltose solution before use.

   NOTE: After performing one experiment, the unused portions of the above media and broth can be stored at 2-8°C for subsequent experiments.

Procedure:

Day 1:

1. Open the vial containing culture and resuspend the culture with 0.25 ml of LB broth.

2. Pick up a loopful of culture and streak onto LB agar plate.

3. Incubate overnight at 37°C.

Day 2:

1. Inoculate a single colony from the revived plate in 25 ml TBM (Tryptone Broth with Maltose). Grow the cells at 37°C till the A_{590} (optical density) reaches ~0.5-0.7.

Day 3:

1. Take seven collection tubes and label them as 1, 2, 3, 4, 5, 6 and 7. Pipette 1.0 ml of SM buffer in tube no. 1 and 900 µl of SM buffer in rest of the tubes.

2. Take 10 µl of stock phage lysate in tube no. 1. Hence the dilution in this tube is 10^{-2}. Mix thoroughly by vortexing and transfer 100 µl to tube no. 2. The dilution in tube no. 2 is 10^{-3}.

3. Vortex tube no. 2 and transfer 100 µl to tube no. 3 to get a dilution of 10^{-4}. Repeat this step until the stock lysate is diluted to10^{-8}.

4. Take seven more collection tubes and label them from 1 to 7. Also take seven LB agar plates and label them from 1 to 7.

5. Take 300 µl of bacterial culture in all seven tubes. Add 100 µl of the respective phage dilution, mix and keep at RT for 20 minutes.
   Note: Mix gently by tapping, do not vortex.

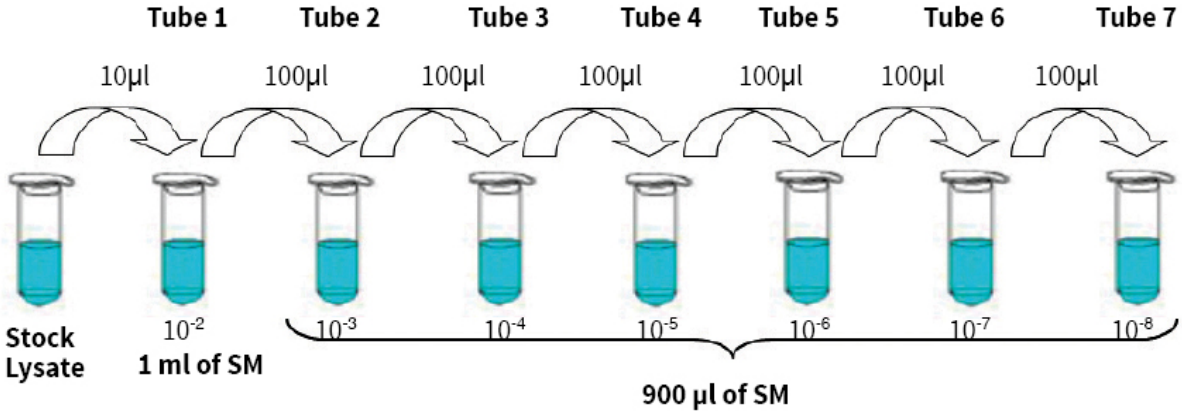


Fig 2: Serial dilution of Phage lysate

6. Take seven sterile test tubes and label them from 1 to 7.

7. Take 4 ml of soft agar in test tube no. 1 and pipette out the entire mixture from collection tube no. 1 from step 6 into the soft agar, mix well and pour the suspension as quickly as possible onto LB agar plate labeled as no.1 and allow it to solidify.

   Note: Temperature of the molten soft agar must be 40-50 °C as either cells will die at higher temperature or agar will solidify at lower temperature. Make sure that the LB agar plates are moisture free.

8. Repeat step 7 for each dilution. After the soft agar has solidified, incubate the plates overnight at 37°C.

Observation and Result:

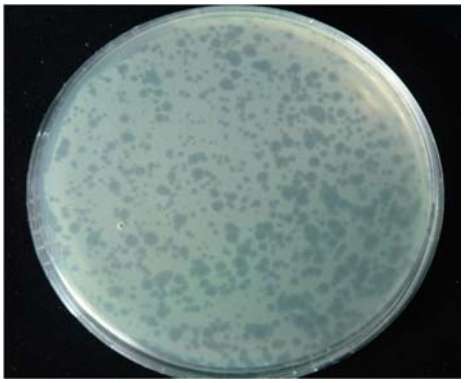


Fig 3: Plaques observed after infecting *E.coli* with phage lambda

Check the plates for clear and distinct plaques. Count the number of plaques for each dilution and note down the results as per Table 2.

Table 2: Results of the phage titration assay

Tube No.	Dilutions	Number of plaques	Phage titre value
1			
2			
3			
4			
5			
6			
7			

Formula for calculating the phage titre value:

Phage titre value = Number of plaque forming units/ml of lysate

For example, if the number of plaques observed at dilution 10^{-5} is 50 then the phage titre value per ml of lysate will be:

Phage titre value = 50 x 10^5 / 100 µl
= 5 x 10^7 / ml

Interpretation:

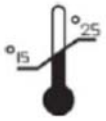
When the phage lysate is 10^{-2} times diluted, distinct plaques are not seen due to the complete lysis of the host. At 10^{-3} dilution, confluent plaques are observed. At lower dilutions clear, distinct and countable plaques are observed. Therefore, at higher dilutions of phage lysate, distinct and countable plaques are formed.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Numbers of plaques do not correlate with the phage dilution	Dilution of the phage is not done properly	Vortex every tube thoroughly before pipetting. Make sure that the tip is changed for every dilution
2	No clear plaques observed on the LB plates	Host cells have died before plating	Temperature of the soft agar should not be more than 45ºC
3	Distinct and clear plaques are not observed	The LB agar plates contain moisture	Make sure that the LB agar plates are completely dry before performing the experiment

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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