

HiPer[®] Bacterial Growth Curve Teaching Kit

Product Code: HTM001

Number of experiments that can be performed: 10

Duration of Experiment:

Day 1: Preparation of media and revival of strain

Day 2: Inoculation of culture

Day 3: Protocol and plotting of graph

Storage Instructions:

- The kit is stable for 6 months from the date of receipt
 - Store *E. coli* culture at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)



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Index

Sr. No.	Contents	Page No.
1	Aim	3
2	Introduction	3
3	Principle	3
4	Kit Contents	4
5	Materials Required But Not Provided	4
6	Storage	4
7	Important Instructions	4
8	Procedure	5
9	Observation and Result	5
10	Interpretation	6
11	Troubleshooting Guide	6

Aim:

To study different growth phases of bacterial population and plot a bacterial growth curve.

Introduction:

Most bacteria reproduce by an asexual process called binary fission, which results in doubling of the number of viable bacterial cells. In binary fission, events occur in sequences i.e. the chromosome duplicates, the cell elongates, and the plasma membrane pinches inward to the center of the cell. The time required for a cell population to divide and double in number is called the generation time or the doubling time. Generation time varies with organism and environment and can range from 20 minutes for a fast growing bacterium under ideal conditions, to hours in not so ideal conditions. The standard bacterial growth curve describes various stages of growth of pure culture of bacteria, beginning with the addition of cells in sterile media to the death of the cells.

The phases of growth typically observed include:

- Lag phase
- Exponential (log, logarithmic) phase
- Stationary phase
- Death phase (exponential or logarithmic decline)

In standard bacterial growth curve one keeps track of cell growth by some measure or estimation of cell number.

Principle:

Bacterial growth refers to an increase in the number of cells. When bacteria are inoculated into a liquid medium and the cell population is counted at intervals, it is possible to plot a typical bacterial growth curve that shows the growth of cells over time. It shows four distinct phases of growth.

- **Lag phase:** During the lag phase, the bacterial cells adapt themselves to the new environment as they are introduced into a new medium. The bacterial population increase in size but show no change in number as they prepare themselves for cell division. They synthesize new enzymes to utilize new nutrients. The length of the lag phase is determined by the characteristics of the bacterial species and by conditions in the media, both the medium from which the organisms are taken and the one to which they are transferred. The bacterial population then enters an active phase of growth called the logarithmic phase.
- **Log or exponential phase:** During this phase, the bacteria divide exponentially. The mass of each cell increases rapidly followed by reproduction. The number of bacteria doubles during each generation time. The growth curve is linear during this phase and the cells are metabolically most active. As the amount of nutrients decrease, the vigor of the population changes and the rate of reproduction and death equalizes. The bacterial population then enters a plateau called as stationary phase.
- **Stationary phase:** During the stationary phase, as the nutrients get exhausted, and there is a build-up of waste and secondary metabolic products, the growth rate decreases to a point where it almost equals to the death rate, hence viable cell number remains same. If incubation is still continued the population then enters the death phase.
- **Decline or death phase:** During the death phase, viable cell population decreases exponentially as the amount of waste products increases. The death of cells in the population exceeds the formation of new cells. This continues until the population is diminished to a tiny fraction of the more resistant cells or the population might die out entirely.

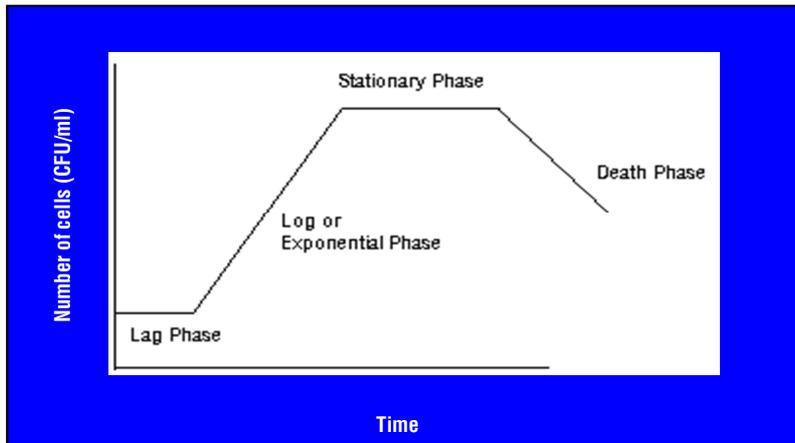


Fig 1: Graphical representation of an ideal bacterial growth curve

Kit Contents:

The kit can be used to study different growth phases of a bacterial population over a period of time in a suitable growth medium.

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	TKC028	<i>E. coli</i> culture	1 No.	2-8°C
2	M1245	Luria Bertani (LB) Broth	75 g	RT
3	MB053	Agar Powder, Bacteriological	1.5 g	RT

Materials Required But Not Provided:

Glass wares: Conical flasks, Measuring cylinder, Sterile test tubes, Sterile Petriplates

Reagents: Distilled water

Other requirements: Incubator, Shaker, Spectrophotometer, Micropipettes, Tips, Sterile Loops

Storage:

HiPer[®] Bacterial Growth Curve Teaching Kit is stable for 6 months from the date of receipt without showing any reduction in performance. On receipt, store *E. coli* culture 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) broth (260 ml):** Suspend 6.5 grams of LB Broth in 260 ml distilled water. Heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
3. **Preparation of LB (Luria Bertani) agar plates (100 ml):** Dissolve 2.5 g of LB media and 1.5 g of agar in 100 ml of sterile distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 °C. and pour on sterile petriplates.

Day 1:

1. Using sterile flexi loop, streak a loopful of *E. coli* culture from the stab onto the LB agar plate. Incubate at 37°C for 18-24 hours.

Day 2:

1. Pick up a single colony of each strain from the LB agar plate and inoculate it into a test tube containing 10 ml of autoclaved LB broth. Incubate the test tube overnight at 37°C.

Day 3:

1. Take 250 ml of autoclaved LB Broth in a sterile 500 ml conical flask.
2. Inoculate 5 ml of the overnight grown culture in above flask.
3. Take OD at zero hour. Incubate the flask at 37°C.
4. Aliquot 1 ml of the culture suspension at an interval of every 30 minutes and take the optical density (OD) at a wavelength of 600 nm using spectrophotometer, till the reading becomes static.

Note: Alternatively, 50-100 µl of formaldehyde can be added to all the 1 ml aliquots of culture suspension taken after every 30 minutes. Optical density of all the aliquots can be taken at the end of the experiment.

5. At the end of experiment, plot a graph of time in minutes on X axis versus optical density at 600nm on Y axis to obtain a growth curve of Escherichia coli.

Observation and Result:

Take the optical density (OD) at regular time intervals as shown in table 2.

Table 2: Optical density obtained for every aliquot of culture suspension

Sr. No.	Time in minutes	Optical Density (OD) at 600 nm
1	00	
2	30	
3	60	
4	90	
5	120	
6	150	
7	180	
8	210	
9	240	
10	270	
11	300	
Read O.D. till absorbance becomes static		

Plot a graph of time in minutes (X axis) versus optical density at 600nm (Y axis). Find out the slope.

Formula for calculating growth rate constant (μ), generation time (g) and growth rate:

1. Growth rate constant (μ)= slope x 2.303
2. Generation time (doubling time) $g = 0.693 / \mu$
Generation time is expressed in hours.
3. Growth rate= 1/generation time

Interpretation:

By performing this experiment, the different stages of bacterial growth curve can be observed. In the lag phase the OD remains constant. With time the cells enter the log phase where the OD increases until the cells reach the stationary phase, where the OD remains more or less constant. If OD is taken for longer period of time (7-8 hours) one can observe a decrease in the value. The generation time and growth rate of the organism in the given media can also be measured from the growth curve.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Log phase observed for long period of time	Nutrient in the media are not getting exhausted	Inoculate more cells
		Accurate temperature not maintained during inoculation and procedure	Always maintain ideal temperature of 37°C while growing the cells
2	Contamination observed	Proper aseptic measures not taken	Ensure that the inoculation and procedure is done aseptically

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