HiPer® PCR Application Teaching Kit

Product Code: HTBM030

Number of experiments that can be performed: 5/20

Duration of Experiment

Protocol: 1.5 hours
Agarose Gel Electrophoresis: 1hour

Storage Instructions:

- The kit is stable for 6 months from the date of receipt
- Store PCR reagents, I00 bp DNA Ladder, Test Samples, Template DNA at -20°C
- Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To demonstrate the direct application of PCR for diagnostic purpose in detecting a specific pathogen.

Introduction:

Polymerase Chain Reaction (PCR) is an *in vitro* method of enzymatic synthesis of specific DNA fragment developed by Kary Mullis in 1983. PCR is used to amplify a precise fragment of DNA from a complex mixture of starting material called as template DNA. It is a very sensitive and specific method for amplification based detection of genes. For this reason this method has wide application in the field of clinical and forensic research purpose. DNA or RNA of pathogenic organisms can be detected by PCR and is the basis for clinical diagnostic tests.

Principle:

Polymerase Chain Reaction (PCR) is a very sensitive and specific method for amplification based detection of genes. The three steps of a successful PCR reaction include Denaturation, Annealing and Extension. The double-stranded DNA melts and forms single stranded DNA at high temperature (Denaturation). Sequence-specific primers bind to the target sequence on single-stranded DNA at lower temperature (Annealing). Taq DNA Polymerase adds dNTPs onto the single stranded DNA at intermediate temperature (Extension). These 3 steps of PCR are usually repeated between 25 to 40 times in each PCR assay. This is a very simple technique for characterizing, analyzing and synthesizing DNA from virtually any living organism (plant, animal, virus, bacteria). Over the years PCR technology has become an essential part of diagnostic research and clinical medicine is getting benefitted from it. Through PCR a specimen of genetic material is copied repeatedly and a sufficient amount of test sample is generated in which the presence of a pathogen can be detected. These PCR-based detection methods are more sensitive as compared to the traditional antibody-based serological diagnostic methods where the infected body's immune response is measured to a pathogen. All the PCR-based clinical tests are able to detect the presence of responsible pathogen much earlier compared to the serologically-based methods, as the infected patients may take weeks to develop antibodies against the infecting pathogen and an earlier detection of infection leads to earlier treatment.

The HiPer® PCR Application Teaching Kit is designed for detection of specific sequence of a specific gene (450 bp) for *E.coli* O157. Conventional PCR testing can provide rapid, sensitive and specific detection of *E.coli* O157. This kit is a qualitative conventional PCR kit which contains the amplification of *E. coli* O157 specific gene using specific primers. The amplified target is detected by using agarose gel electrophoresis. Gel electrophoresis is used to analyze the amplification of desired gene region for target pathogen based on separation of DNA fragments according to their size.

Kit Contents:

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

Sr. No.	Product Code	Materials Provided	Quantity		Storage
			5 expts	20 expts	
1	TKC380*	10X Assay Buffer with 10mM dNTP mix and 25mM $MgCl_2$	0.22 ml	0.7 ml	-20°C
2	TKC377*	Control PCR Product of EC O157	0.07 ml	0.23 ml	-20°C
3	TKC378*	Primer mix	0.03 ml	0.1ml	-20°C
4	TKC381*	Test Sample 2	0.015 ml	0.05 ml	-20°C
5	MBT049	I00 bp DNA Ladder	0.02 ml	0.07 ml	-20°C
6	TKC120*	Taq DNA Polymerase (5 U/ µl)	0.01ml	0.025 ml	-20°C
7	TKC379*	Test Sample 1	0.015 ml	0.05 ml	-20°C
8	ML065	Nuclease Free water	0.5 ml	1.5 ml	R T
9	MB002	Agarose	6 g	18 g	R T
10	ML016	50X TAE	60 ml	250 ml	R T
11	ML015	6X Gel Loading Buffer	0.05 ml	0.2 ml	2-8 °C
12	CG282	Polypropylene Tubes, 0.2 ml (PCR Tubes)	15 Nos.	50 Nos.	R T

*** Always give a quick spin before opening the vial as the liquid material may stick to the wall or to the cap of the vial**

Materials Required But Not Provided:

Glassware: Measuring cylinder, Beaker
Reagents: Ethidium bromide (10 mg/ml)
Other requirements: Thermocycler, Electrophoresis apparatus, UV Transilluminator, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Microwave/ Hotplate/ Burner, Crushed ice

Storage:

HiPer® PCR Application Teaching Kit is stable for 6 months from the date of receipt without showing any reduction in performance. On receipt, store Test Samples, 100 bp DNA Ladder and all PCR reagents at -20°C and 6X Gel Loading Buffer should be stored at 2-8°C. Other reagents can be stored at room temperature (15-25°C).

Important Instructions:

- Read the entire procedure carefully before starting the experiment.
- Keep all the solutions in the icebox during use.
- The 100bp DNA ladder supplied in the kit is ready to use and can be directly loaded onto the agarose gel.

Procedure:

1) **Preparation of master mix for PCR**

Take two PCR tubes; add the following ingredients in mentioned order:

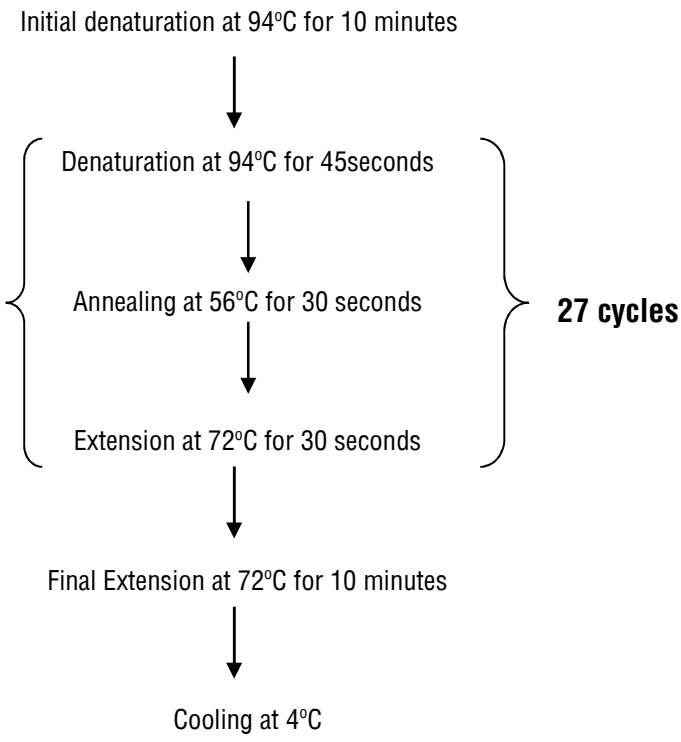
	Test Sample 1	**Test Sample 2**
10X Assay Buffer with 10mM dNTP mix and 25mM $MgCl_2$	15 µl	15 µl
Taq DNA Polymerase	0.5 µl	0.5 µl
Test Sample 1	2 µl	0
Test Sample 2	0	2 µl
Primer mix	2 µl	2 µl
Nuclease free water	30.5 µl	30.5 µl
Total volume	**50 µl**	**50 µl**

2) Tap the tubes for 1 – 2 seconds to mix the contents thoroughly.

3) Place the tubes in the thermo cycler block and set the program to get DNA amplification.

PCR Amplification Cycle:

Carry out the amplification in a thermo cycler for 27 cycles using the following reaction conditions.



Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 1.5 % agarose gel, add 0.75 g agarose to 50ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate, swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool down to about 55-60ºC. Add 0.5µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

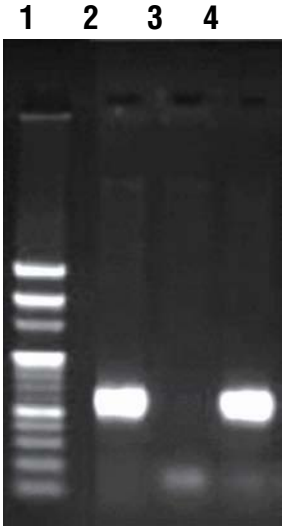
Loading of the PCR Samples: Load 3 µl of ready to use DNA ladder into the first well. Add 2 µl of 6X Gel loading buffer to 10 µl of PCR products. Load the PCR samples into the following two wells. In the next well load 10 µl EC O157 control PCR products.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black-Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

* Molecular biology grade water is recommended (Product code: ML024).

Observation and Result:

After completion of PCR, perform agarose gel electrophoresis of the amplified products with the ladder and check for the test sample that shows amplification at the same size as that of the control PCR product.



Lane 1: 100bp DNA Ladder
Lane 2: PCR for Test Sample 1
Lane 3: PCR for Test Sample 2
Lane 4: Control PCR product of *E. coli* O157

Interpretation:

After performing agarose gel electrophoresis, one can detect the presence of the pathogen *E. coli* O157 in one of the test sample. The other test sample does not have *E. coli* O157 infection as it is not giving any amplified product with the primer specific to of *E. coli* O157.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	No or poor amplification yield	Template or dNTPs may be degraded, enzymes may have been inactive	Store the kit at -20°C and avoid repeated freeze thaw. Also keep all the materials in ice while performing the experiment
		Thermoycler operation or program improper	Ensure proper functioning of Thermocycler. Run positive control with every reaction
		Inadequate mixing of the reaction tube	Mix the reaction mixture using a micropipette, avoid air bubble
2	Smearing of the product	DNA degraded	Work in sterile conditions to avoid contamination. Avoid vigorous mixing of the DNA samples
3	Primer-Dimer observed	Concentration of primers and dNTPs may be inappropriate	Use recommended concentration of primers and dNTPs

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