HiPer® Restriction Modification Teaching Kit

Product Code: HTBM020

Number of experiments that can be performed: 10

Duration of Experiment

Protocol: 3 hours
Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of receipt
- Store Lambda DNA, Restriction Enzymes, CpG Methyltransferase, SAM and Assay Buffer 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 study the restriction modification system using restriction enzyme and its methyl transferase.

Introduction:

Prokaryotic restriction-modification (R-M) systems were first recognized in *Escherichia coli* nearly 50 years ago and are now known to be ubiquitous among bacterial species. In general, R-M systems consist of two distinct enzymatic activities: first, a restriction endonuclease that cleaves DNA at a specific recognition sequence, and second, a DNA methyltransferase that methylates DNA at the same site and thus prevents cleavage by the cognate restriction enzyme. Restriction–modification systems allow bacterial cells to distinguish between their own DNA and any foreign DNA entering the cell, and to destroy the latter. They operate through two enzyme activities: a restriction endonuclease that cleaves the foreign DNA, and a modification methyltransferase that protects the host DNA.

Principle:

Phage (or viruses) invade all types of cells. Bacteria are one favorite target. Defense mechanisms have been developed by bacteria to defend themselves from these invasions. The system they possess for this defense is the restriction modificiation system. This system is composed of a restriction endonuclease enzyme and a methylase enzyme and each bacterial species and strain has their own combination of restriction and methylating enzymes.

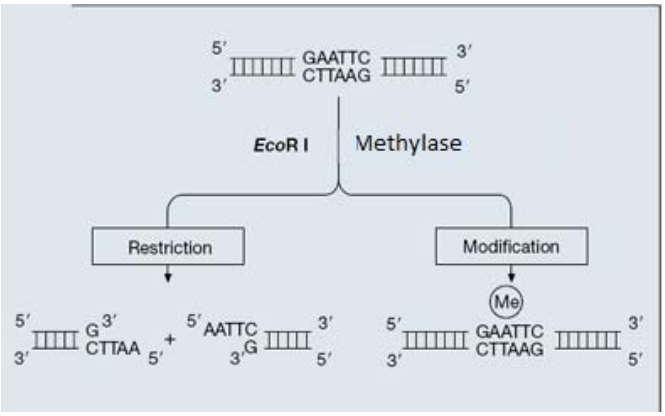

Fig 1: Target site of restriction endonuclease modifies upon methylation by Methyl transferase enzyme

The restriction-modification system is used to protect bacteria from invasion by viral DNA. The bacterial cell uses the restriction enzyme to cut the invading DNA of the virus at the specific recognition site of the enzyme. This prevents the virus from taking over the cellular metabolism for its own replication. But bacterial DNA will also contain sites that could be cleaved by the restriction enzyme which have to be protected. This protection is offered by the action of the methylase. The methylase recognizes the same target site as the restriction enzyme and adds a methyl group to a specific nucleotide in the restriction site. Methylated sites are not substrates for the restriction enzyme. The bacterial DNA is methylated immediately following replication so it will not be a suitable substrate for restriction endonuclease cleavage. But it is unlikely that the invading viral DNA will have been methylated so it will be an appropriate target for cleavage. Thus, the viral DNA is ***restricted*** in the bacterial cell by the restriction enzyme, and the bacterial DNA is ***modified*** by the methylase and is provided protection from its own restriction enzyme.

Kit Contents:

The kit demonstrates restriction modification of Lambda DNA.

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	TKC411	Lambda DNA	0.2 ml	-20°C
2	TKC412	Assay Buffer	0.140 ml	-20°C
3	TKC413	SAM	0.140 ml	-20°C
4	TKC414	CpG Methyl transferase	0.035 ml	-20°C
5	ML024	Molecular Biology Grade Water	1 ml	RT
6	*MBRE020	Restriction Enzyme Mlu I	0.025 ml	-20°C
7	*MBRE011	Restriction Enzyme Pst I	0.025 ml	-20°C
8	ML016	50X TAE	120 ml	R T
9	ML015	6X Gel Loading Buffer	0.4 ml	2-8°C
10	MB002	Agarose	6 g	R T
11	CG281	Polypropylene Tubes (0.5 ml)	60 No	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:

Glass wares: Measuring cylinder, Beaker
Reagents: Ethidium bromide (10 mg/ml), Distilled Water or Molecular Biology Grade Water
Other requirements: Crushed ice, Electrophoresis apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Crushed ice, Microwave/ Hotplate/ Burner

Storage:

HiPer® Restriction Modification Teaching Kit is stable for 12 months from the date of receipt without showing any reduction in performance. On receipt, store the Restriction Enzymes, CpG Methyltransferase, Assay Buffers, Lambda DNA and SAM at -20ºC. 6X Gel Loading Buffer should be stored at 2-8ºC. All other kit contents can be stored at room temperature (15-25ºC).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. The restriction enzymes, CpG Methyltransferase, SAM and Assay Buffer are temperature sensitive and should always be placed on ice during the experiment.
3. While performing the experiment place the Lambda DNA, restriction enzymes, CpG Methyltransferase, SAM and Assay Buffer on ice.
4. Use fresh tip while adding different solution to the tube.
5. While preparing the reaction mixture the enzymes should always be added at last.

Procedure:

1. Before starting the experiment, crush ice and place the vials containing Lambda DNA, restriction enzymes, CpG Methyltransferase, SAM and Assay Buffer onto it.
2. Take 6 Nos. of 0.5 ml tubes and label as 1,2,3,4,5 and 6.
3. To the labelled vials, add components as indicated in the following table.

	Tube 1	**Tube 2**	**Tube 3**	**Tube 4**	**Tube 5**	**Tube 6**
Lsmbda DNA	3 µl	3 µl	3 µl	3 µl	3 µl	3 µl
Assay Buffer	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
SAM	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
CpG Methyltransferase	-	1 µl	-	1 µl	-	1 µl
Milli Q water	13 µl	12 µl	12 µl	12 µl	13 µl	12 µl
Incubate at 37°C for 2 hours						
Mlu I	-	-	1 µl	1 µl	-	-
Pst I	-	-	-	-	1 µl	1 µl
Milli Q water	1 µl	1 µl	-	-	-	-
Incubate at 37°C for 1 hour						

4. Stop the reaction of all the 6 tubes by adding 3 µl of 6X Gel Loading Buffer.
5. Run the samples on agarose gel as given below.

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% agarose gel, measure 0.5 g agarose in a glass beaker or flask and add 50ml 1X TAE buffer. Heat the mixture on a microwave or hot plate or burner, 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 to about 55-60ºC. Add 0.5 µl 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.

NOTE: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

Loading of the DNA samples: Load all the samples in the order 1 to 6 on to the gel.

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

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

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV Transilluminator.

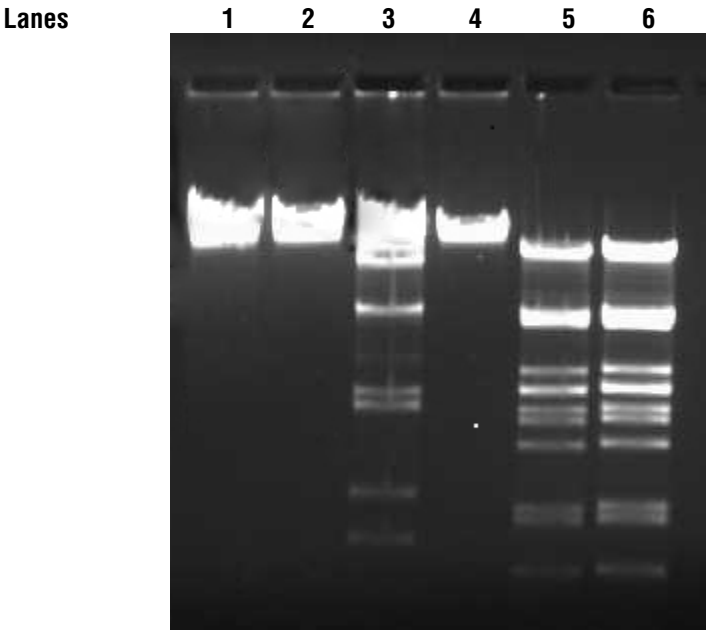

Fig 2: Restriction Modification of Lambda DNA

Lane 1: Lambda DNA
Lane 2: Lambda DNA + CpG Methyltransferase
Lane 3: Lambda DNA + Mlu I
Lane 4: Lambda DNA + CpG Methyltransferase + Mlu I
Lane 5: Lambda DNA + Pst I
Lane 6: Lambda DNA + CpG Methyltransferase + Pst I

After running the samples on agarose gel, look for the digestion pattern for the two restriction enzymes.

Interpretation:

1. **Lane 1**: An Intact band of undigested λDNA is observed, because no CpG Methyltransferase or restriction enzymes added.
2. **Lane 2:** An Intact band of undigested λDNA is observed, because no CpG Methyltransferase is added and no restriction enzymes.
3. **Lane 3:** Lambda DNA digested with Mlu I since it was not methylated with CpG Methyltransferase
4. **Lane 4:** Lambda DNA is not digested with Mlu I since it was methylated with CpG Methyltransferase which has protected the DNA from digestion by Mlu I.
5. **Lane 5:** Lambda DNA digested with Pst I. No methylation has been done.

6. **Lane 6:** Lambda DNA digested with Pst I, even after the DNA has been methylated with CpG Methyltransferase. This clearly shows that the methylating sites and the digestion sites are different for the two different enzymes Mlu I and Pst I.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	Improper results	Components of the reaction mixture not mixed properly	Ensure that all the components are thoroughly mixed by gentle pipetting.
		Reagents intermixed	Always use fresh and sterile tips. Do not interchange tips with the buffers and enzymes
		Degradation of restriction enzymes	Always place the vials containing restriction enzymes on ice as they are temperature sensitive
		Reaction mixture incubated for longer time than specified in the protocol	Do not exceed the incubation time.
2	Improper resolution of bands on agarose gel	Gel not run for sufficient duration	Run the gel for longer period of time till the bands are separated properly

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