HiPer® Plasmid DNA Extraction Teaching Kit (Column Based)

Product Code: HTBM002

Number of experiments that can be performed: 10/20

Duration of Experiment: 3 days

Day 1: Revival of Host
Day 2: Inoculation of culture
Day 3: Plasmid Extraction and Agarose Gel Electrophoresis

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Control DNA and Ampicillin at -20°C
- Store 6X Gel Loading Buffer, *E.coli* cells (with plasmid) and Resuspension Solution at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

Isolation and purification of plasmid DNA (using spin column).

Introduction:

Plasmid is an extra-chromosomal DNA molecule different from the chromosomal DNA which is capable of independent replication. The term "Plasmid" was first introduced by the American molecular biologist Joshua Lederberg in 1952. Plasmid size varies from 1 to over 1000 kilo base pairs (kbps). Plasmids are mostly circular and double-stranded. Plasmids are found in a wide variety of bacterial species; but are sometimes found in eukaryotic organisms e.g., in *Saccharomyces cerevisiae.*

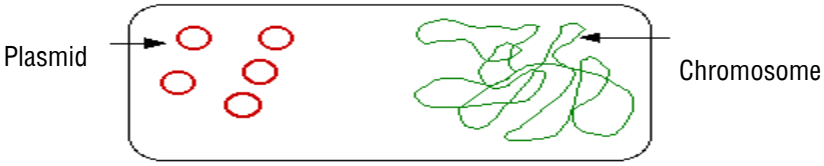


Fig 1: A bacterium showing that the plasmids are not part of the chromosomal DNA

Plasmids typically have two important elements:

- An origin of replication
- A selectable marker gene (e.g. resistance to ampicillin)

Conformations of plasmid: Plasmid DNA may appear in one of the five conformations which are as follows:

- **"Nicked Open-Circular"** DNA has one strand cut.
- **"Relaxed Circular"** DNA is fully intact with both strands uncut, but has been enzymatically "relaxed" (supercoils removed).
- **"Linear"** DNA has free ends, either because both strands have been cut, or because the DNA was linear *in vivo*.
- **"Supercoiled"** (or "Covalently Closed-Circular") DNA is fully intact with both strands uncut, and with a twist built in, resulting in a compact form.
- **"Supercoiled Denatured"** DNA is like supercoiled DNA, but has unpaired regions that make it slightly less compact.

The conformations listed above are in order of electrophoretic mobility from slowest to fastest; and for a given size, run at different speeds in the gel during electrophoresis.

Plasmid DNA Extraction Teaching Kit (Column Based) provides a fast and easy method for purification of total DNA for reliable applications in PCR, Library screening and Sequencing, Restriction digestion etc. It is fast, simple and efficient method and does not contain harmful organic compounds such as phenol and chloroform.
The DNA purification procedure using the miniprep spin columns comprises of three steps:

- Adsorption of DNA to the membrane
- Removal of residual contaminants
- Elution of pure plasmid DNA

HiElute Miniprep Spin Column format allows rapid processing of samples. The columns have a high binding capacity and high quality DNA is obtained.

Principle:

The bacterial culture is grown overnight in suitable medium and harvested. The harvested bacterial culture is then lysed so that plasmid can come into solution and collected by centrifugation after which it is applied on to the silica column for specific binding of the DNA molecules in the presence of high salt concentration. HiElute Miniprep Spin Column eliminates the need for alcohol precipitation, expensive resins and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. The adsorbed DNA is washed to remove contaminants, and the pure plasmid DNA is eluted in Elution Buffer. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, *in vitro* translation, blotting and sequencing.

Kit Contents:

This kit can be used to perform plasmid DNA extraction from *E.coli* strain using spin column.

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	20 expts	
1	TKC003	Control DNA	0.11 ml	0.22 ml	-20ºC
2	TKC006	*E. coli* cells (with plasmid)	1 No.	1 No.	2-8ºC
3	DS0020	Resuspension solution (HP1)	3 ml	6 ml	2-8ºC
4	DS0021	Lysis solution (HP2)	3 ml	6 ml	RT
5	DS0022	Neutralization solution (HN3)	4 ml	8 ml	RT
6	DS0032	Wash Solution (HPB)	6 ml	12 ml	RT
7	DS0024	Wash solution II	8 ml	16 ml	RT
8	DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	0.6 ml	1.2 ml	RT
9	MB002	Agarose	4.8 g	9.6 g	RT
10	DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	10 Nos.	20 Nos.	RT
11	PW1139	Collection Tubes, Polypropylene (2.0 ml)	20 Nos.	40 Nos.	RT
12	DBCA016	Collection Tubes(Uncapped), Polypropylene (2.0 ml)	10 Nos.	20 Nos.	RT
13	M1245	Luria Bertani (LB) Broth	5 g	10 g	RT
14	MB053	Agar Powder, Bacteriological	1 g	2 g	RT
15	MB104	Ampicillin	0.025 g	2 X 0.025 g	-20ºC
16	ML016	50X TAE	120 ml	240 ml	RT
17	ML015	6X Gel Loading Buffer	0.05 ml	0.1 ml	2-8ºC

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Reagents: Distilled water, Ethidium bromide (10 mg/ml)
Other requirements: UV Spectrophotometer, Tabletop microcentrifuge (with rotor for 2.0 ml tubes), Electrophoresis apparatus, Incubator Shaker, UV Transilluminator, Micropipettes, Vortex Mixer, Shaker, Tips, Adhesive tape, Crushed ice, Microwave / Hotplate

Storage:

HiPer® Plasmid DNA Extraction Teaching Kit (Column Based) is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Control DNA and Ampicillin at -20°C. Store *E. coli* cells (with plasmid), 6X Gel Loading Buffer and Resuspension solution 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. Thaw all refrigerated samples before use.
3. Thoroughly mix the reagents. Examine the solutions for any kind of precipitation. If any solution (except Resuspension Solution) forms a precipitate warm at 55-65°C, until the precipitate dissolves completely, allow it to cool to room temperature before use.
4. Ensure that clean & dry eppendorf tubes and tips are used for the procedure.
5. **Ampicillin Preparation:** Dissolve 25 mg of the Ampicillin antibiotic in 500 µl of sterile distilled water to prepare a stock concentration of 25 mg/500 µl. Store at -20°C.
6. **Preparation of Luria Bertani Broth (10 ml):** Dissolve 0.25 g of Luria Bertani broth in 10 ml of distilled water and autoclave.
7. **Preparation of LB (Luria Bertani) agar plates with ampicillin (50 ml):** Dissolve 1.25 g of LB media and 0.75 g of agar in 50 ml of distilled water. Sterilize by autoclaving and allow the media to cool down to 40-45 ° C. Add 50 µl of ampicillin into it and pour on sterile petriplates.

Procedure:

Read the important instructions before starting the experiment.

Day 1: Revival of Strain

1. Open the vial containing culture and resuspend the cells with 0.25 ml of LB broth.
2. Pick up a loopful of culture and streak onto LB agar plate with ampicillin.
3. Incubate overnight at 37°C.

Day 2: Inoculation of Culture

1. Pick up a single colony from LB agar plate and inoculate in 10 ml of LB broth containing 10 µl ampicillin.
2. Incubate the test tube overnight at 37°C for 300 rpm.

Day 3: Plasmid DNA Extraction

1. **Harvest Cells**
   Take 1.5 ml of the overnight grown culture into a collection tube and centrifuge the cells at 13,000 rpm for 1 minute. Discard the supernatant culture medium.

   NOTE: For good plasmid yields, the $O.D_{600}$ of the culture should be around 3.0×10^6 cells/ml.

2. Resuspend Cells

Resuspend the bacterial pellet in 250 µl of Resuspension Solution and mix well by gentle vortexing till no cell clumps are visible.

3. Lyse Cells

Add 250 µl of Lysis Solution to lyse the cells. Mix thoroughly by gently inverting the tube 4-6 times.

NOTE: Do not vortex the tubes as it may result in the shearing of plasmid DNA which may contaminate the plasmid DNA. Do not allow this lysis reaction to exceed more than 5 minutes.

4. Neutralize

Add 350 µl of Neutralization Solution and immediately mix thoroughly by inverting the tube 4-6 times.

NOTE: On addition of Neutralization Solution, the mixture should become cloudy and the precipitation should be homogeneous.

4. Centrifuge the sample at 13,000 rpm for 10 minutes to obtain a compact white pellet.

6. Load lysate on HiElute Miniprep Spin column (Capped) [DBCA03]

Carefully remove the supernatant and transfer it to a collection tube containing HiElute Miniprep Spin column (capped) provided and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through liquid.

7. First Wash

Add 500 µl of Wash Solution (HPB) to the HiElute Miniprep Spin column placed in the collection tube (uncapped) and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through liquid.

8. Second Wash

Add 700 µl of Wash Solution II to the HiElute Miniprep Spin column placed in the collection tube (uncapped) and centrifuge at 13,000 rpm for 1 minute.

9. Discard the flow-through liquid and centrifuge the tube with HiElute Miniprep Spin column for an additional one minute to remove the traces of Wash Solution.

10. DNA Elution

Transfer the HiElute Miniprep Spin column to a clean new uncapped collection tube (DBCA016) and add 50 µl of the Elution Buffer and allow it to stand for 1 minute at room temperature followed by centrifugation for 1 minute at 13,000 rpm.

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer, then centrifuge. Elution with volumes less than 50 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

11. Transfer the eluate to a fresh capped 2ml collection tube (PW1139) for longer DNA storage.

Storage of the eluate with purified DNA: The eluate contains pure plasmid DNA. For short term storage (24-48 hours) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturation of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

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: Add 0.4g agarose in 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture in 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 55-60°C. 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: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10 µl of DNA sample. Mix well by pipetting and load the sample into the well. Load the Control DNA after extracting the DNA sample.

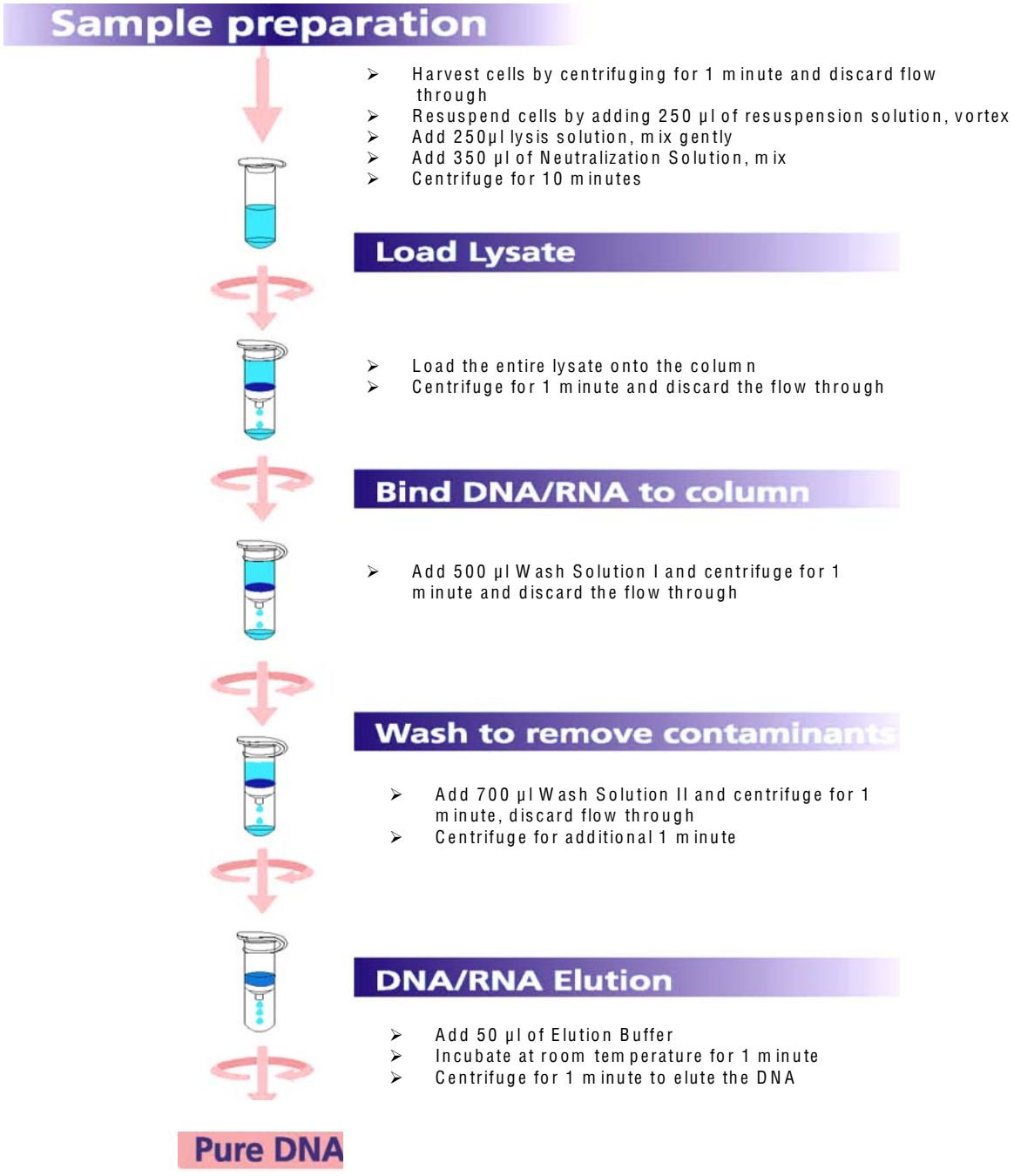
Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.
* Molecular biology grade water is recommended (Product code: ML024).

Quantitation of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of DNA is calculated by the following formula:

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Flowchart:



Note: **All centrifugation steps should be carried out at 13,000 rpm**

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV Transilluminator and calculate the yield and purity using UV Spectrophotometer.

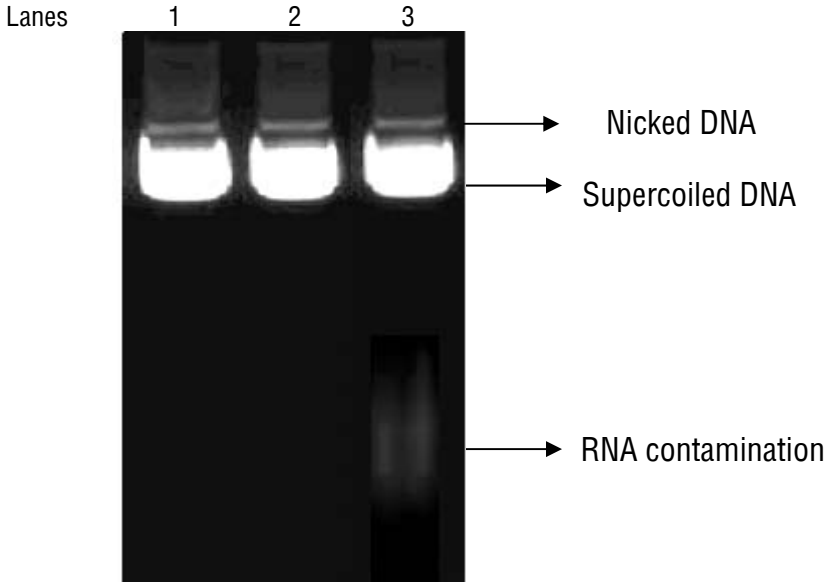


Fig 2: Gel image of isolated plasmid DNA

Lane 1: Control DNA
Lane 2: Extracted Plasmid DNA
Lane 3: Plasmid DNA with RNA contamination

Table 2: Absorbance of the extracted plasmid DNA at 260 nm and 280 nm

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula:

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Interpretation:

On analyzing plasmid DNA after electrophoresis, two bands were observed.

1) Nicked DNA

2) Supercoiled DNA

The supercoiled DNA being more compact runs faster than the nicked form. The nicked form runs slowly since their open structure experiences more resistance while passing through the gel matrix. These are seen as bands above the supercoiled form.

The data in lanes 1 and 2 demonstrate that highly purified plasmid DNA has been obtained with no visible RNA contamination when electrophoresed on agarose gel. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic DNA as shown in lane 3. RNA being of lower molecular weight runs faster than the genomic DNA. RNA contamination is observed when the RNase treatment has not been carried out properly.

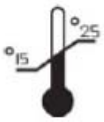
An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. If the A_{260}/A_{280} ratio is 1.6-1.9, then the isolated DNA sample is considered to be pure. If higher A_{260}/A_{280} ratio is observed it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sr. No	Problem	Possible Cause	Solution
1	Poor or low plasmid DNA recovery	Antibiotic activity is insufficient	Use a fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are light sensitive and degrade during long term storage at 2-8ºC
		Residual supernatant from cell media	Remove the supernatant after the initial centrifugation; the remaining supernatant can be removed by an additional centrifugation
		Alkaline lysis is prolonged	The lysis time should not exceed more than 5 minutes
2	Additional band seen ahead of supercoiled plasmid during gel electrophoresis	A portion of the plasmid DNA is permanently denatured	Do not allow the lysis reaction to exceed 5 minutes NOTE: The nicked or covalently open double-stranded plasmid DNA runs slower than the super coiled DNA during electrophoresis.
3	Poor performance in downstream enzymatic applications	Purification is incomplete	Salts in one or more of the solutions may have precipitated. Examine the solutions for any kind of precipitation, if any solution forms a precipitate warm at 55-65°C until the precipitate dissolves completely; allow it to cool to room temperature before use

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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