MB505 HiPurA® Bacterial Genomic DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB505		
		20 Preps	50 Preps	250 Preps
DS0014	Gram Positive Lysis Solution (GPLA)	6 ml	15 ml	75 ml
DS0015	Lysis Solution (AL)	5.6 ml	14 ml	70 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0031	Prewash Solution (PWB)	12 ml	30 ml	150 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
DS2280	Proteinase K	10 mg	25 mg	125 mg
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DS2281	Lysozyme	0.28 gm	0.7 gm	3.5 gm
DBCA03	HiElute Miniprep Spin Column(Capped) [in DBCA16 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tube(Uncapped) , Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from bacterial cultures

Introduction

HiPurA® Genomic DNA Purification Kits provide a fast and easy method for purification of total DNA for reliable applications in PCR, Southern blotting technique etc. The DNA purification procedure using the miniprep spin columns comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR amplification and Southern blotting.

HiPurA® Bacterial Genomic DNA Purification Kit

This kit simplifies isolation of DNA from bacteria (Gram positive and Gram negative) by the spin-column procedure. Bacterial cells are grown in a medium till they reach log phase and are harvested by centrifugation. After harvesting, the bacterial (Gram positive) cell wall is degraded by lysozyme and Proteinase K. For Gram negative bacteria, the lysozyme treatment is not required. Following lysis, the DNA is bound to the silica-gel membrane of the HiElute Miniprep Spin Column (Capped) to yield approximately upto 20 µg of pure DNA.

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Two rapid wash steps remove trace amount of salt and protein contaminants resulting in the elution of pure DNA in the Elution Buffer provided with the kit.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA. The use of spin column facilitates the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously. This 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. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit. The purified DNA is upto 20-30 kb in length and can be used for further downstream applications.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. A single elution with 200 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 200 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size upto 20-30 kb, and is suitable for direct use in PCR, restriction digestion, and Southern blotting applications.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer (ET) 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 for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The A_{260}-A_{320} /A_{280}-A_{320} ratio should be 1.6-1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by HiPurA® Bacterial Genomic DNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

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

Materials needed but not provided

- 37°C water bath or heating block
- 55°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2 ml tubes)
- Ethanol (96 - 100%)
- Lysostaphin (Optional) (For *Staphylococcus* species only)
- Mutanolysin (Optional) (For *Streptococcus* species only)
- Molecular Biology Grade Water (Product code: ML064)

Storage

Store the HiPurA® Bacterial Genomic DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.

2. Preheat a water bath or heating block to 37°C (for Gram positive bacteria only).

3. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

4. Ensure that clean & dry tubes and tips are used for the procedure.

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	4 ml	16 ml
50	10 ml	40 ml
250	50 ml	200 ml

6. **Reconstitute Proteinase K (DS2280)**

   The HiPurA® Bacterial Genomic DNA Purification Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of Proteinase K is 33.5 units/mg dry weight.

   Resuspend the Proteinase K (DS2280) powder in Molecular Biology Grade Water (ML064) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
20	10 mg	0.5 ml
50	25 mg	1.25 ml
250	125 mg	6.25 ml

   The product as supplied is stable at room temperature (15-25°C); upon reconstitution store at -20°C as mentioned in storage instructions.

   NOTE: The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solution for storage.

7. **Prepare Lysozyme Solution (Product Code: DS2281) [For Gram positive bacteria only]**

   Prepare a 45 mg/ml solution of Lysozyme (approximately 2.115 x 10^6 unit/ml) with the Gram Positive Lysis Solution (GPLA) as the diluent, which is provided in the kit. Lysozyme solution should be freshly prepared prior to use.

   Example: In order to make 1 ml of Lysozyme solution, dissolve 45 mg of lysozyme (provided) in 1 ml of Gram Positive Lysis Solution (GPLA). Pipette the mixture up and down or vortex to dissolve the lysozyme.

   NOTE: Lysozyme dissolves readily by pipetting up and down as opposed to vortexing. Excessive vortexing may cause foaming.

   For each DNA preparation, 200 µl of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at -20°C.

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research. RNase A (e.g., bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3' end of unpaired C and U residues.

Unit Definition for RNase A
One unit of the enzyme causes an increase in absorbance of 1.0 at 260nm when yeast RNA is hydrolyzed at 37°C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit. It is completely free of DNases and proteases. The specific activity is 90 U/mg.

The product as supplied is stable at room temperature (15-25°C).

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature (15-25°C) and are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5}\, r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Specimen Collection and Handling

Collect overnight culture from sterile flask with the help of micropipette. Store the remaining culture at 2-8°C for short term use.

Types of Specimen: Bacterial culture

Procedure

A. Gram Negative Bacterial Preparation

1. **Harvesting of cells**
   Pellet 1.5 ml of an overnight bacterial broth culture in provided 2 ml capped collection tube by centrifuging for 2 minutes at 13,000rpm at room temperature (15-25°C). Remove the culture medium and discard.

   NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column.

2. **Resuspend cells**
   Resuspend the pellet thoroughly in 180 µl of Lysis Solution (AL) (DS0015).

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to the sample. Mix and incubate for 30 minutes at 55°C. If residual RNA is not a concern, continue with step 4.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 4.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds) and incubate at 55°C for 10 minutes. Continue with step C (Prepare for binding).

   NOTE: A homogeneous mixture is essential for efficient lysis.

B. <u>Gram Positive Bacterial Preparation</u>

1. Prepare Lysozyme Solution using Lysozyme from chicken egg white, which is provided in the kit. Prepare a 45 mg/ml stock solution of lysozyme as described under General Preparation Instructions. 200 µl of Lysozyme Solution is required per isolation procedure. Prepare extra solution to account for pipetting error.

   NOTE: (Optional) For higher yields - If working with *Staphylococcus* species, supplement the Lysozyme Solution with 200 units/ml of lysostaphin. For *Streptococcus* species, supplement the Lysozyme Solution with 250 units/ml of mutanolysin.

2. **Harvest Cells**
   Pellet 1.5 ml of bacterial broth culture in provided 2 ml capped collection tube by centrifuging for 2 minutes at 13,000 rpm at room temperature (15-25°C). Remove the culture medium completely and discard.

   NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column.

3. **Resuspend cells**
   Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step 1 of Gram Positive Bacterial Preparation) and incubate for 30 minutes at 37°C.

4. **Lyse cells**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to the sample. If residual RNA is not a concern continue with step 5.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 5.

5. Add 200 µl of Lysis Solution (C1) (DS0010). Vortex thoroughly for few seconds and incubate at 55°C for 10 minutes, then continue with step C (Prepare for binding).

   NOTE: A homogeneous mixture is essential for efficient lysis.

<u>DNA ISOLATION FROM GRAM POSITIVE AND GRAM NEGATIVE BACTERIA</u>

This is a continuation of the procedure from the lysates prepared in procedure A and B.

C. Prepare for binding
   Add 200 µl of ethanol (95-100%) to the lysate and mix thoroughly by vortexing for few seconds.

   NOTE: A homogenous mixture is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the HiElute Miniprep Spin Column. This precipitate does not interfere with the DNA isolation procedure or with any subsequent application. Do not use alcohols other than ethanol because this may result in reduced yields.

D. Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]

Transfer the lysate obtained from step C onto HiElute Miniprep Spin Column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute at room temperature (15-25°C). Discard the flow-through liquid and place the spin column in same 2 ml collection tube.

NOTE: Use a wide bore pipette tip to reduce shearing of the DNA while transferring contents onto the column. It is essential to apply all of the precipitate to the column. If the solution has not completely passed through the membrane, spin at a higher speed until all the solution has passed through. Centrifugation at full speed will not affect the yield or purity of the DNA.

E. Prewash

Add 500 µl of Prewash Solution to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute at room temperature (15-25°C). Discard the flow-through liquid and re-use the same collection tube with the column.

F. Wash

(Prepare diluted Wash Solution (DS0012) as indicated in General Preparation Instructions)

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge for 3 minutes at 13,000 rpm at room temperature (15-25°C). Discard the flow through and spin again at same speed for the additional 1 minute to dry the column. The column must be free of ethanol before eluting the DNA.

G. DNA Elution

Transfer the HiElute Miniprep Spin Column (Capped) to fresh uncapped collection tube. Pipette 200 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute at room temperature (15-25°C) to elute the DNA.

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volumes less than 200 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.

H. Transfer the eluate to a fresh capped 2 ml collection tube for longer storage.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) 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 denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Warning and Precautions

For Research Use only. Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

Each lot of HiMedia's HiPurA® Bacterial Genomic DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Bacterial cells (1.5 ml)	Upto 20 µg	1.6-1.9

References

1. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA , 1993).

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Lysozyme is difficult to dissolve	Solution is inadequately mixed	Pipette repeatedly to dissolve the lysozyme as opposed to vortexing; as vortexing will cause foaming and reduce lysozyme solubility. Lysozyme may not dissolve readily; complete dissolution is not needed prior to use as it will dissolve during the 37°C incubation.
2.	HiElute Miniprep Spin Capped Column is clogged	Sample volume is large	Use smaller quantity of sample; to salvage the current preparation, clogging can be alleviated by increasing the g force or spinning for longer time until the lysate passes through the spin column.
3.	Lysate appears to be gelatinous prior to loading onto the column	Sample volume is large	Use fewer cells (≤ $1x10^{10}$ cells/ml). The incubation time and or the amount of Proteinase K solution or Lysozyme solution can be increased, depending on which procedure is performed i.e. for Gram positive or Gram negative bacteria. Eg: The incubation time and the amount of enzyme can be doubled.
4.	Poor / Lower yield of genomic DNA	Sample is old	Yield of genomic DNA varies from different species and strains of bacteria.

			It is necessary to use cells before they reach their maximum density or they become confluent.
		Incomplete lysis of cells	The incubation time and or the amount of Proteinase K solution or Lysozyme Solution can be increased, depending on which procedure is performed i.e. for Gram positive or Gram negative bacteria. eg: The incubation time and the amount of enzyme can be doubled.
		Lysate/Ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the column.
		DNA elution is incomplete. Eluate contains residual ethanol from the wash	DNA yield can be improved by incubating the Elution Buffer for 5 minutes at room temperature (15-25°C) after it is added to the column. Ethanol from the final wash should be eliminated completely before eluting DNA. Spin the tubes for longer time to dry the column completely.
		Wash Solution Concentrate was not diluted before use	Check that the Wash Solution Concentrate is properly diluted with ethanol as per instructions.
		Use of water instead of Elution Buffer for elution of DNA.	Elution Buffer is recommended for optimal yields and storage of the genomic DNA. If water is used instead of the Elution Buffer the pH should be at least 7.0 to avoid acidic conditions, which may cause acid hydrolysis of DNA when stored for long periods of time. (**NOTE:** Only DNase/RNase and Protease free water should be used for eluting DNA)

5.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is low	Background reading is high due to silica fines	The DNA sample can be centrifuged at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
		Sample diluted in water	Use either Elution Buffer provided, or (10 mM Tris-HCl, 0.5 mM EDTA pH 9.0) or 10 mM Tris-HCl pH 8.0-8.5 as the eluant.
6.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too high.	RNA contamination	RNase A treatment should be included in future isolations or the final product can be treated with RNase A and repurified.
7.	DNA is sheared	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, mix with gentle pipetting or invert until homogenous, instead of vortexing as it reduces shearing of DNA considerably.
		Cells are old	Cells grown for a longer time period may lyse prematurely when subjected to cell wall lysing enzymes, which may result in the release of endogenous nucleases and subsequent DNA degradation.
8.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	After the washing steps, the eluate should not come in contact with the column. Spin the column for 1 minute at maximum speed (12,000-16,000 x g) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The HiElute Miniprep Spin Capped Column should be transferred to a new 2.0 ml collection tube before adding the wash solution.

Please refer disclaimer Overleaf.

Safety Information

HiPurA® Bacterial Genomic DNA Purification Kit is for laboratory use only; not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical assistance

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

Symbols

	Manufacturer		Do not use if package is damaged
LOT	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)	REF	Catalogue number

Identification No.: PIMB505
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Disclaimer :

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