MB545 HiPurA® MT DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB545		
		20 Preps	50 Preps	250 Preps
DS0015	Lysis Solution (AL)	12 ml	30 ml	150 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]	4 ml	10 ml	50 ml
MB086	Proteinase K	12 mg	30 mg	150 mg
MB098	Lysozyme	600 mg	1.5 g	7.5 g
DBCA04	HiBead Tubes	20 nos.	50 nos.	250 nos.
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from human sputum, cells and tissues samples.

Introduction

HiPurA® MT DNA Purification Kit provides a fast and easy method for purification of DNA from *Mycobacterium* spp. for reliable applications in PCR and 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 down stream applications such as restriction enzyme digestion, PCR amplification and Southern blotting.

HiPurA® MT DNA Purification Kit

This kit simplifies isolation of DNA from *Mycobacterium* spp. from bacteria and tissue samples by the spin-column procedure. Bacterial cells are grown in the medium till they reach log phase and are harvested by centrifugation.

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CE

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Samples are subjected to Proteinase K digestion. Following lysis is the binding of DNA to the silica-gel membrane of the HiElute Miniprep Spin column (Capped) to yield approximately upto 20 µg of pure DNA. Two rapid wash steps removes 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 50 kb (predominant fragment size 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 50-100 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 100 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size up to 50 kb (predominant fragment size 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® MT 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:

- 70°C water bath or heating block
- 80°C Oven
- 37°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96- 100%)
- Molecular biology grade water (Product Code: ML024)
- Tissue Homogenizer
- 0.85% Saline

Storage

Store the HiPurA® MT DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or heating block to 70°C.
2. Preheat a water bath or heating block to 37°C.
3. Preheat the heating block or oven at 80°C.
4. **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.
5. Ensure that clean & dry tubes and tips are used for the procedure.
6. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

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

7. **Reconstitute Proteinase K (MB086)**
   The HiPurA® MT 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 (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
20	12 mg	0.6 ml
50	30 mg	1.5 ml
250	150 mg	7.5 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.

   If the isolated DNA is to be used for PCR, mix with gentle pipetting or inversion until homogenous, instead of vortexing in the following procedure as it reduces shearing of DNA considerably.

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 260 nm 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 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 Handling and Collection

Collect sputum sample in sterile container (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the sputum sample is at room temperature (15-25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: sputum sample, cells and tissues

Procedure

NOTE: Follow decontamination protocol MB545D- HiPurA® Decontamination Kit for MTB before proceeding with MB545- HiPurA® MT DNA Purification Kit

1. **Sample Preparation**
   For sputum:
   Follow decontamination protocol MB545D- HiPurA® Decontamination Kit for MTB**.** Add 1ml of treated sputum sample to a new collection tube, polypropylene (2.0ml) and proceed with step b.

   For cells:
   Pellet 5 ml of L J slant suspension by centrifuging for 3 minutes at 4,000x g (≈6,500 rpm). Remove the culture medium and discard. Proceed to step 2.

   For Tissues:
   Homogenize upto 20-30 mg of tissue sample in 1 ml of 0.85% saline using tissue homogenizer (Product code: GW174). Pellet the sample at 13,000 rpm and discard the supernatant. Proceed to step 2.

2. **Resuspend cells**
   Resuspend the pellet thoroughly in 250 µl of Lysis Solution (AL) (DS0015).

3. Incubate at 80°C oven for 60 minutes.

4. **Lysis**
   To the HiBead Tube (DBCA04) provided, add the above resuspended *Mycobacterium* spp. suspension. Mix by vortexing.

5. Secure the HiBead tube horizontally on a flat-bed vortex pad using a tape and vortex at maximum speed for 10 minutes.

6. Ensure that the HiBead tubes rotate freely in your centrifuge without rubbing. Centrifuge the tube at 13,000 x g (≈14,000 rpm) for 1 minute at room temperature.

   NOTE: Make sure not to exceed the speed more than 13,000 x g or else the tubes may break.

7. Transfer the supernatant to a 2.0 ml capped collection tube.

8. **Prepare for cell lysis**
   Add 25 mg of Lysozyme (MB098) and 25 µl of the Proteinase K solution (20 mg/ml) **(Refer to General Preparation instructions)** to the sample. Mix and incubate for 2 hours at 37°C. If residual RNA is not a concern continue with step 9.

   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 9.

9. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds), and incubate at 70°C for 10 minutes.

   NOTE: A homogeneous mixture is essential for efficient lysis.

10. **Prepare for binding**
    Add 200 µl of ethanol (95-100%) to the lysate and mix thoroughly by vortexing for 15 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 (Capped). 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.

11. **Load lysate in HiElute Miniprep Spin column (Capped) [DBCA03]**
    Transfer the lysate obtained from step 6 into HiElute Miniprep Spin column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the spin column in a same 2.0 ml uncapped collection tube.

    NOTE: Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column. It is essential to apply all of the precipitate to the HiElute Miniprep Spin column. If the solution has not completely passed through the membrane, centrifuge again 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.

12. **Prewash**
    Add 500 µl of Prewash Solution (PWB) (DS0031) to the HiElute Miniprep Spin column (Capped) and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

13. **Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WS) to the column and centrifuge for 3 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm). The column must be free of

ethanol before eluting the DNA. Therefore centrifuge the column for the additional 1 minute at maximum speed if residual ethanol is seen.

14. **DNA Elution**
    Transfer the HiElute Miniprep Spin Column (Capped) to new uncapped 2.0ml collection tube provided. Pipette 50-100 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≥10,000 rpm) for 1 minute to elute the DNA.

    Optional: A second elution can be collected by repeating step 14.

15. Transfer the eluate to a fresh capped 2ml collection tube for long term storage.

    NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer (ET), then centrifuge. Elution with volumes less than 100 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.

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

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical 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

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Sputum sample	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.	HiElute Miniprep	Sample volume is	Use smaller quantity of sample, to

	Spin column (Capped) is clogged	large	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. The yield of genomic DNA reduces.
2.	Lysate appears to be gelatinous prior to loading onto the column	Sample volume is large	Use fewer cells (≤ 1x 10^{10} cells /ml) or upto 30mg of tissue sample. The incubation time and or the amount of Proteinase K solution can be increased.
3.	Poor / Lower yield of genomic DNA	Sample is old	Yield of genomic DNA varies from different species and strains of *Mycobacteria*. It is necessary to use cells before they reach their maximum density or they become confluent.
4.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is low.	Incomplete lysis of cells	The incubation time and or the amount of Proteinase K solution can be increased.
		Lysate / Ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 sec 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
		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.
5.	Purity of the DNA is lower than expected; A_{260}/A_{280}	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.

	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.
6.	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.
7.	Downstream applications are inhibited.	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.
		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 column (Capped) should be transferred to a new 2.0 ml collection tube before adding the wash solution in steps 7 and 8.

Safety Information

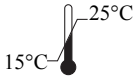
The HiPurA® MT DNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing 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

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
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