

## MB504 HiPurA® Blood Genomic DNA Miniprep Purification Kit

### Kit Contents

Product Code	Reagents provided	MB504		
		20 Preps	50 Preps	250 Preps
ML116	Resuspension Solution (1X PBS)	6 ml	15 ml	75 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0011	Prewash Solution Concentrate (PW)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
MB086	Proteinase K	10 mg	25 mg	125 mg
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 Tubes(Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

### Intended Use

Recommended for isolation of DNA from human blood, plasma and serum samples.

### Introduction

HiPurA® Blood Genomic DNA Miniprep Purification Kit provides a fast and easy method for purification of total DNA for downstream applications such as PCR, Southern blotting technique etc. The DNA purification procedure using the miniprep spin column 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 genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

### HiPurA® Blood Genomic DNA Miniprep Purification Kit

This kit simplifies isolation of DNA from fresh, old (more than 24 hours) and frozen blood with spin-column procedure. Genomic DNA purification from blood involves cell lysis, which is achieved by incubation of whole blood in a solution containing chaotropic ions in the presence of Proteinase K at 55°C. HiElute Miniprep Spin Column (Capped) contains specially developed membranes for optimal binding of genomic DNA. After the initial binding of DNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA is finally eluted in the Elution Buffer provided with the kit. Typical yield is 4-12 µg of total DNA from 200 µl of whole blood.

### **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 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. An elution with 200 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reactions. 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® Blood Genomic DNA Miniprep 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**

- 55°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)

#### **Storage**

Store the HiPurA® Blood Genomic DNA Miniprep 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 55°C
2. **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. The reagent should be at room temperature (15-25°C) before use.
3. Ensure that clean & dry tubes and tips are used for the procedure.
4. **Dilute Prewash Solution Concentrate (PW) (DS0011) as follows:**

Number of Preps	Prewash Solution Concentrate (PW)	Ethanol (96-100%)
20	6 ml	9 ml
50	15 ml	22.5 ml
250	75 ml	112.5 ml

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	6 ml	18 ml
50	15 ml	45 ml
250	75 ml	225 ml

6. **Reconstitute Proteinase K (MB086)**

The HiPurA® Blood Genomic DNA Miniprep 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	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; 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 solutions for storage.

## 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 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 whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (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 blood 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: Whole blood, Plasma, Serum

### **Procedure**

#### **1. Collect Blood**

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

**For Frozen blood:** To 200 µl of frozen blood pellet (kept on ice), add 200 µl of Lysis Solution (C1) (DS0010). Thaw the pellet with continuous pipetting and proceed with step 2 for Proteinase K and RNase A treatment (optional). Incubate at 55°C for 10 minutes and then proceed to step 4 of the protocol.

**NOTE:** When extracting DNA from frozen blood, it is very important that the blood should be kept on ice and directly mixed with Lysis Solution (C1). Do not allow the frozen blood pellet to thaw except when it is directly mixed with Lysis Solution (C1). This prevents release of apoptotic enzymes that can decrease the DNA yield drastically.

**NOTE:** If the sample is less than 200 µl, add the Resuspension solution (ML116) to bring the volume upto 200 µl.

2. Add 20 µl of the Proteinase K solution (20 mg/ml) (DS0013) into 2.0 ml capped collection tube containing 200 µl of the whole blood. Vortex for 10-15 seconds to ensure thorough mixing.

#### **Optional RNase A treatment**

If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003). Vortex for 10-15 seconds and incubate for 2 minutes at room temperature (15-25°C); continue with step 3.

**3. Lysis reaction**

Add 200 µl of the Lysis Solution (C1) (DS0010) to the sample, vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 55°C for 10 minutes.

**NOTE:** If cell clumps are visible, the sample can be mixed gently by pipetting to obtain a homogenous mixture.

**4. Prepare for Binding**

Add 200 µl of ethanol (96-100%) to the lysate obtained from the above step for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting.

**NOTE:** A homogenous solution is essential.

**5. Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Transfer the lysate obtained from step 4 into the spin column provided. Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

**6. Prewash**

**(Prepare Prewash Solution as indicated in General Preparation Instructions)**

Add 500 µl of diluted Prewash Solution to the column and centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

**7. Wash**

**(Prepare Wash Solution as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution to the column and centrifuge at  $12,000-16,000 \times g$  ( $\approx 13,000-16,000$  rpm) for 3 minutes to dry the column. Discard the flow-through liquid and spin the empty column for another minute at the same speed if residual ethanol is observed. Discard the collection tube containing the flow through liquid and place the column in a new uncapped 2.0 ml collection tube.

**NOTE:** The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

**8. DNA Elution**

Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at  $\geq 6,500 \times g$  ( $\approx 10,000$  rpm) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

**NOTE:** DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (DNA yield would be low). Storing DNA in water may cause acid hydrolysis. 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 volume less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

**9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA 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**

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
200 µl whole blood	4-12 µg	1.6-1.9

**References**

1. Sambrook, J., *et al.* Molecular Cloning: A laboratory Manual, 2<sup>nd</sup> 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).

**Trouble shooting Guide:**

Sr.No.	Problem	Possible Cause	Solution
1.	Presence of cell clumps / colored residue on the spin column after washing	Inefficient cell lysis due to improper mixing of the lysis buffer with the blood sample	The sample and the Lysis Solution (C1) should be mixed thoroughly by pulse-vortexing.
		Due to decreased Proteinase K activity	Do not add Proteinase K directly to the Lysis Solution (C1). Ensure that the stock solution is stored as indicated.
2.	Poor or low genomic DNA recovery	Sample is old or degraded	DNA yield varies among fresh or old (more than 24 hrs old) samples. Whole blood should be used within a few hours of collection for best results. (Whole blood can be stored at 4°C for at least 3 months).

		Lysate/ethanol mixture is not homogenous	In order to obtain a homogenous solution, mix thoroughly by gentle pipetting before adding to the HiElute Miniprep Spin Column (Capped).
		DNA elution is improper	Ensure that the DNA elution is in 200 µl of Elution Buffer (ET) as mentioned in step 8. To improve the DNA yield incubate for 5 minutes at room temperature (15-25°C) after it is added to the column.
		Eluate contains residual ethanol from wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for an additional 2 minutes to dry the membrane completely. In order to avoid the interference of ethanol, fresh tube can be used for elution.
		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer (ET) is recommended for optimal yield 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.  <b>NOTE:</b> Only DNase/RNase and Protease free water should be used for eluting DNA.
3.	Purity of the DNA is lower than expected ( $A_{260}/A_{280}$ ratio is less)	Blood sample was older than 24 hours	Prewash Solution should be used in the first wash step for old blood samples.
		Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute and use the supernatant to repeat the absorbance readings.
		Eluate was diluted in water for absorbance measurement	Use the Elution Buffer (ET) provided with the kit.
		Purification is incomplete due to column overloading or inadequate lysis	Reduce the initial volume of the sample or increase the lysis time while monitoring the lysis visually.
4.	$A_{260}/A_{280}$ 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.
5.	Shearing of genomic DNA	The blood sample used is old, degraded or has undergone repeated freeze/ thaw cycles	If the blood sample is old, the eluate may yield degraded DNA. For best results, fresh whole blood should be used or whole blood stored at 4°C for up to 3 months.

**Safety Information**

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**

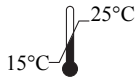
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In vitro diagnostic medical device



CE Marking



Storage temperature



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