MB516 HiPurA® Blood Genomic DNA Midiprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB516	
		10 preps	25 preps
ML116	Resuspension Solution (1X PBS)	30 ml	75 ml
DS0010	Lysis Solution (C1)	34 ml	85 ml
DS0011	Prewash Solution Concentrate (PW)	12 ml	30 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	7 ml	17.5 ml
MB086	Proteinase K	44 mg	110 mg
DBCB01	HiPure Midiprep Spin Column (in PW144 Collection Tube)	10 nos	25 nos
PW144	Collection Tube (15 ml conical)	20 nos	50 nos

Intended Use

Recommended for isolation of DNA from human blood samples.

Introduction

HiPurA® Genomic DNA Purification Kits provide a fast and easy method for purification of total DNA for reliable applications in PCR, Library screening and Sequencing. The DNA purification procedure using the midiprep columns comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia's HiPure Midiprep Spin Column format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, ligation, PCR and sequencing.

HiPurA® Blood Genomic DNA Midiprep 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. HiPure Midiprep Spin Column 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 15-50 µg of total DNA from 1-2 ml of whole blood.

HiPure Midiprep Spin Column (DBCB01)

HiPure Midiprep Spin Column is based on the advanced silica binding principle presented in a spin 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.

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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. Purified DNA upto 20 - 30 kb in length can be obtained using HiPurA® Blood Genomic DNA Midiprep Purification Kit which 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. Elution with 300 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 300 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 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 Midiprep 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
- Tabletop Centrifuge (with rotor for 15.0 ml tubes) capable of ≥ 3,500 x g (5,000 rpm). **Swinging bucket rotor is recommended over fixed angle rotor**.
- Ethanol (96 – 100%)
- Optional - Molecular Biology Grade Water (Product code: ML024)
  - RNase A Solution (20 mg/ml) (Product code: DS0003)

Storage

Store the HiPurA® Blood Genomic DNA Midiprep 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. **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.

3. Ensure that clean & dry collection 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%)
10	12 ml	18 ml
25	30 ml	45 ml

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
10	8 ml	24 ml
25	20 ml	60 ml

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

The HiPurA® Blood Genomic DNA Midiprep 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 to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular biology grade Water
10	44 mg	2.2 ml
25	110 mg	5.5 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 Solution 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).

The product can be procured from HiMedia: RNase A Solution (20 mg/ml) (Product Code: DS0003)

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

Note

- Volume of solutions mentioned in the procedure steps are for 1.0 - 2.0 ml of whole blood
- Volume of solutions for lower volumes of blood sample i.e. 0.3 - 1.0 ml of whole blood, is mentioned in Table at the end of the procedure.

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

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.

2. Add 200 µl of the Proteinase K solution (20mg/ml) into a 15 ml centrifuge tube (not provided).

3. Add 1.0-2.0 ml of whole blood. Vortex briefly (10-15 seconds) to ensure thorough mixing of enzyme.

   NOTE: Add the Resuspension Solution (1X PBS) (ML116) to bring the volume of sample up to 2.0 ml, if necessary**.** Vortex to ensure thorough mixing. Whole blood may be stored at 4°C for atleast 3 months before preparing the DNA. If residual RNA is not a concern, continue with step 4.

   Optional RNase A treatment (not provided) (Product code DS0003)

   If RNA-free genomic DNA is required, add 200 µl of RNase A solution (20 mg/ml). Vortex (10-15 seconds) to ensure thorough mixing of enzyme and incubate for 2 minutes at room temperature, continue with step 4.

4. **Lysis reaction**
   Add 2.4 ml of the Lysis Solution (C1) (DS0010) to the sample, vortex thoroughly or mix by inverting tube for atleast 1 minute to obtain a homogenous mixture. Incubate at 70°C for 10 minutes.

   NOTE: If cell clumps are visible the sample can be pipetted gently to obtain a homogeneous mixture.

   For Frozen blood: To 1.0-2.0 ml of frozen blood pellet (kept on ice), add 2.4 ml of Lysis Solution (C1) and thaw the pellet with continuous pipetting. Then proceed to step 2 for Proteinase K and RNase A treatment (optional). Incubate at 70°C for 10 minutes and then proceed to step 5 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.

5. **Prepare for Binding**
   Add 2.0 ml of ethanol (96-100%) to the lysate obtained from step 4 for preparation of lysate for binding. Mix thoroughly by inverting the tube few times followed by vortexing for 5-10 seconds.

   NOTE: A homogeneous solution is essential to ensure efficient binding.

6. **Load lysate in HiPure Midiprep Column (DBCB01)**
   Transfer one half of the lysate obtained from step 5 into HiPure Midiprep Column provided. Centrifuge at ≥1850 x g (≥3,000 rpm) for 3 minutes. Discard the flow-through liquid and place the column back into the same collection tube.

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

7. Load the remaining solution from step 5 onto the column and centrifuge at ≥1850 x g (≥3,000 rpm) for 3 minutes. Remove the HiPure Midiprep Column, discard the flow-through, and place the column back into a new 15 ml collection tube.

   NOTE: If the solution has not passed completely through the column membrane, centrifuge again at a higher speed.

8. **Prewash**
   (Prepare Prewash Solution as indicated in General Preparation Instructions)
   Add 2 ml of diluted Prewash Solution to the column and centrifuge at ≥3,500 x g (≥5,000 rpm) for 1 minute. Discard the flow-through, and place the column back into the 15 ml collection tube.

   Optional
   To improve A_{260}/A_{280} ratio, perform an extra wash step by adding 2.0 ml of Ethanol (96-100 %) to the column and centrifuge at ≥3,500 x g (≥5,000 rpm) for 1 minute. Discard the flow-through and place the column back into the 15 ml collection tube. Proceed to step 10 to continue with the protocol.

9. **Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 2 ml of diluted Wash Solution to the column and centrifuge at ≥3,500 x g (≥5,000 rpm) for 15 minutes. Place the column in a new 15 ml collection tube.

   NOTE: The column must be free of ethanol before eluting the DNA. **Incubating the HiPure Midiprep Column for 10 min at 70°C in an incubator to evaporate residual ethanol is recommended.** (If the centrifugal force is below 5,000 rpm, it is strongly recommended to follow the above step).

10. **DNA Elution**
    Pipette 300 µl of the Elution Buffer (ET) (DS0040) or molecular biology grade water (Product code: ML024) directly onto the column without spilling to the sides. Incubate for 5 minutes at room temperature. Centrifuge at ≥3,500 x g (≥ 5,000 rpm) for 2 minutes to elute the DNA.

a. For highly concentrated DNA, continue with step 12a. For maximum DNA yield, continue with step 12b.

b. For maximum concentration, reload the eluate onto the column membrane, incubate at room temperature for 5 minutes. Centrifuge at ≥3,500 x g (≥ 5,000 rpm) for 2 minutes.

c. For maximum yield, pipette additional 300 µl of the Elution Buffer (ET) (DS0040) directly onto the column and incubate for 5 minutes at room temperature. Centrifuge at ≥3,500 x g (≥ 5,000 rpm) for 2 minutes to elute the DNA.

NOTE: Elution with volumes less than 300 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 of -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.

Table: Volumes of solutions to be used in the procedure for sample size of 0.3 – 1.0 ml of whole blood.

Product Code	Reagents provided	Volume of solution to be used in the procedure for sample volume of 0.3-1.0ml of whole blood
DS0010	Lysis Solution (C1)	1.2 ml
-	Ethanol (96-100%)	1.0 ml
DS0011	Prewash Solution Diluted (PW)	2.0 ml
DS0012	Wash Solution Diluted (WS)	2.0 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	300 µl
MB086	Proteinase K Solution (20mg/ml)	100 µl
DS0003	RNase A Solution (20 mg/ml)	100 µl

DNA Concentration

Alcohol precipitation is recommended if a more concentrated plasmid preparation is desired.

Transfer the eluate to a clean 2.0 ml collection tube. Add 0.1 volumes of 3.0 M sodium acetate, pH 5.2 and 0.7 volumes of isopropanol to the recovered genomic DNA. Mix well by inversion and centrifuge at ≥13,000 × g (≈ 14,000 rpm) at 4°C for 30 minutes. Decant the supernatant, being careful not to disturb the pellet. Rinse the pellet with 1.5 ml of 70% ethanol and centrifuge as before for 10 minutes. Carefully decant the supernatant and air-dry the pellet until the residual ethanol has evaporated. Resuspend the DNA pellet in the desired volume of Elution Buffer or molecular biology grade water.

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
Whole blood	15-50 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.	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.
		Hemoglobin can be difficult to remove from the blood of certain animal species (eg. monkey and mouse), and may interfere with downstream applications	Perform extra wash steps with diluted Prewash Solution (PW) (DS0011).
2.	Poor or low genomic DNA recovery	Sample is old or degraded	DNA yield varies among fresh and 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 future use, for at least 3 months).
		Lysate/ethanol mixture is not homogeneous	In order to obtain a homogeneous solution, vortex for about 5-10 seconds before adding to the HiPure Midiprep Column. If the sample is to be used for downstream application such as PCR, mixing by gentle pipetting or inversion is advised.

		DNA elution is improper	Ensure that the DNA elution is in 300 µl of Elution Buffer (ET). To improve the DNA yield, incubate for 5 minutes at room temperature 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 5-10 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. (**NOTE**: 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; use the supernatant to repeat the absorbance readings.
		Eluate was diluted in water for absorbance measurement	Use either the Elution Buffer provided or 10mM Tris-Cl, pH 8.0-8.5.
		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.
		Protein contamination	Perform an extra wash step with 2.0 ml of Ethanol (96-100%), between the Prewash step (step 8) and the Wash step (step 10).
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.
6.	Formation of white precipitate in Lysis Solution (C1)	Storage for long period at low temperatures	Incubate the solution at 56°C to dissolve the precipitate and cool to room temperature (15-25°C) before use.

Safety Information

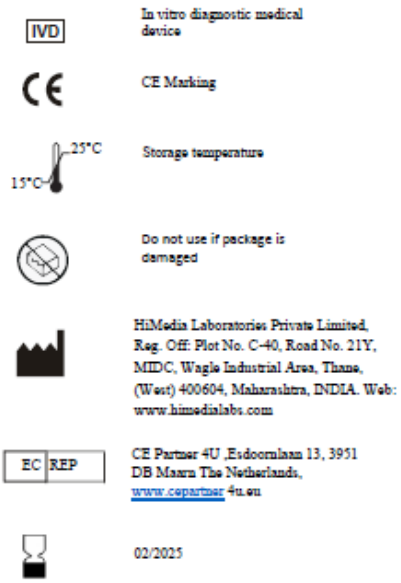
The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

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

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.



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