MB524 HiPurA® Forensic Sample Genomic DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB524		
		20 preps	50 Preps	250 preps
DS0015	Lysis Solution (AL)	8 ml	20 ml	100 ml
DS0010	Lysis Solution (C1)	8 ml	20 ml	100 ml
DS0011	Prewash Solution Concentrate (PW)	8 ml	20 ml	100 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	2 ml	5 ml	25 ml
MB086	Proteinase K	10 mg	25 mg	125 mg
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 250nos

Intended Use

Recommended for isolation of DNA from human blood/animal blood, plasma, serum and other samples such as cells, tissues, stool sample and body fluids.

Introduction

HiPurA® Forensic Sample Genomic DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR and Southern blotting technique. 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 genomic DNA is obtained from various species. The genomic DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA® Forensic Sample Genomic DNA Purification Kit

This kit simplifies isolation of DNA from cigarette butts, envelopes, hair, nail clippings, stamps, or blood, saliva or semen stains with spin-column procedure. Following lysis, DNA binds to the silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield purified DNA. Two rapid wash steps remove trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer (ET) provided with the kit.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

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.

Elution

A single elution with 50 µl of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 50 µl will increase the final DNA concentration, but will reduce the overall DNA yield.

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® Forensic Sample 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

- Heating Block or thermomixer at 55°C
- 70°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- 1M DTT (Dithiothreitol) solution
- Ethanol (96 – 100%)
- Molecular Biology Grade Water (Product code: ML024)

Storage

Store the HiPurA® Forensic Sample Genomic 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. Set the heating block or thermomixer at 55°C.
2. Preheat a water bath or heating block to 70°C.

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 Prewash Solution Concentrate (PW) (DS0011) as follows:**

Number of Preps	Prewash Solution Concentrate (PW)	Ethanol (96-100%)
20	8 ml	12 ml
50	20 ml	30 ml
250	100 ml	150 ml

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	4 ml	12 ml
50	10 ml	30 ml
250	50 ml	150 ml

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

   The HiPurA® Forensic Sample 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.

8. Resuspend the Proteinase K 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

9. 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.

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 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.

Collect cells, tissues, Serum, Plasma, stool sample and body fluids in a clean sterile container and store the samples at 2-8°C for short term storage or -20°C for long term storage. Repeated freeze- thaw of samples should be avoided.

Ensure that the blood/ tissue/ cells/stool/bacterial culture/yeast Culture, Serum, Plasma and body fluids 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, tissues, Serum, Plasma, stool and body fluids.

Procedure

1. **Preparation of Forensic Sample**
   Prepare the following forensic samples according to the steps given below:
   a. Cigarette butts
   b. Envelopes or stamps
   c. Hair roots
   d. Hair shafts without roots
   e. Nail clippings
   f. Material stained with blood, saliva or semen

a. To lyse cigarette butts

Cut a 1cm^2 piece of outer paper from the end of the cigarette or filter. Cut it further into 6 smaller pieces and transfer the pieces into a 2.0ml capped microcentrifuge tube.

b. To lyse envelopes or stamps

Cut a 0.5 - 2.5 cm^2 sample from the envelope or stamp. Cut it further into smaller pieces and transfer the pieces into a 2.0ml capped microcentrifuge tube.

NOTE: Surface contamination can be reduced by using a swab moistened with distilled water, before cutting the envelope or stamp.

c. To lyse hair roots

Cut a 0.5 - 1.0 cm piece starting from the hair bulb. Transfer the piece into a 2.0ml capped microcentrifuge tube.

d. To lyse hair shafts (without roots)

Cut the hair shaft into 0.5-1.0 cm pieces. Transfer the pieces into a 2.0ml capped microcentrifuge tube.

e. To lyse nail clippings

Clip the nail into small pieces. Transfer the pieces into a 2.0ml capped microcentrifuge tube.

f. To lyse material stained with blood, saliva or semen

Take 0.5cm^2 area of the stained material and cut into smaller pieces. Transfer the pieces into a microcentrifuge tube.

2. **Lysis of Forensic Sample**
   Add 300 µl Lysis Solution AL (DS0015), 20 µl Proteinase K (20 mg/ml) **(Refer to General preparation Instructions)** and 20 µl of 1M DTT to the material in the collection tube. Mix thoroughly by pulse-vortexing for 10-15 seconds.

3. Incubate at 55°C for atleast 1- 1½ hours with shaking at 900 rpm in a thermomixer.

   NOTE: If using a heating block or water bath, vortex the tube after every 10 minutes for 10-15 seconds to improve lysis of the sample material.

4. Add 300 µl of Lysis Solution C1 (DS0010) and mix thoroughly by pulse-vortexing for 10-15 seconds.

   NOTE: To ensure efficient lysis, mix the sample and Lysis Solution C1 thoroughly to form a homogenous solution. A white precipitate may form when Lysis Solution C1 is added to Lysis Solution AL. This precipitate will dissolve during incubation in Step 5.

5. Incubate at 70°C for 10 minutes with shaking at 900 rpm in a thermomixer.

   NOTE: If using a heating block or water bath, vortex the tube after every 3 minutes for 10-15 seconds to improve lysis of the sample material.

6. Centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 1 minute at room temperature.

7. **Prepare for Binding**
   Transfer the supernatant carefully, obtained from step 6, into a new 2.0 ml collection tube (not provided) and add 300 µl of ethanol (96- 100%) for preparation of the lysate for binding. Mix thoroughly by vortexing for 5-10 seconds.

8. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Add 650 µl of the mixture from step 7, including any precipitate, which may have formed, to the HiElute Miniprep Spin Column (Capped). Centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature. Discard the flow-through.

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

9. Repeat step 8 with the remaining sample. Discard the flow-through liquid.

10. **Prewash**
    (Prepare the Prewash Solution (PW) as indicated in General Preparation Instructions)
    Place the column in a same 2.0 ml collection tube and add 700 µl of diluted Prewash Solution (PW) (DS0011) to the column. 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.

11. **Wash**
    (Prepare the Wash Solution (WS) as indicated in General Preparation Instructions)
    Add another 700 µl of diluted Wash Solution (WS) (DS0012) to the HiElute Miniprep Spin Column (Capped) and centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 1 minute at room temperature. Discard the flow-through liquid and reuse the same collection tube.

12. Centrifuge the column for 2 minutes at 20,000 x g (≈14,000 rpm) to dry the column membrane to remove the traces of residual ethanol, if observed. Place the column in a new uncapped 2.0 ml collection tube.

13. **DNA Elution**

    Pipette 20 - 50 µl of the Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the centre of the HiElute Miniprep Spin Column (Capped) membrane 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.

    NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) 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.

14. 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.

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)

Warning and Precautions

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

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

Performance and Evaluation

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

Quality Control

Type of Sample	DNA Yield	DNA Purity
Hair roots	2-15 µg of DNA	1.6-1.9
Hair shafts	2-15 µg of DNA	1.6-1.9
Blood	2-15 µg of DNA	1.6-1.9

Troubleshooting guide

Sr. No.	Problem	Possible Cause	Solution
1.	Spin column is clogged	Lysate/ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain homogenous solution before applying it to the column. If minimally sheared DNA is required for downstream applications like PCR, mix with gentle pipetting or inversion until homogenous instead of vortexing.
		DNA elution is improper	Ensure that the DNA elution is in 50 µl of Elution Buffer. To improve the DNA yield incubate for 5 minutes at room temperature after Elution Buffer is added to the column.
		Ethanol was omitted during binding	Ensure that ethanol is added in step 7 before adding the sample to the HiElute Miniprep Spin Column (Capped) in step 8.
		Eluate contains residual ethanol from the wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for additional 2 minutes to dry the membrane completely. In order to avoid the interference of ethanol, always use a fresh tube for elution.
		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 free water should be used for eluting DNA.
2.	Purity of the DNA is lower than expected;	Eluate was diluted in water for absorbance measurement.	Use either the Elution Buffer provided or 10 mM Tris-HCl, pH 8.0.

	(A_{260}/A_{280} ratio is low)	Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
3.	A_{260}/A_{280} ratio is too high	RNA contamination	RNase A treatment can be included in future isolations or the final product can be treated with RNase A Solution and repurified.
4.	Shearing of genomic DNA	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, instead of vortexing mix with gentle pipetting or invert until homogenous. This reduces shearing of DNA considerably.
5.	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 2 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm) if necessary, after emptying the collection tube.
		Salt is carried over in the final eluate containing DNA	The column should be transferred to a new 2.0 ml collection tube before adding the wash solution in steps 10-11.

Safety Information

The HiPurA® Forensic Sample Genomic 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.

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

12/2024

PIMB524_0/1221 MB524-08

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: techhelp@himedialabs.com Website: www.himedialabs.com