

MB573

HiPurA[®] Urine DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB573		
		20 Preps	50 Preps	250 Preps
ML116	Resuspension Solution (1X PBS)	20 ml	50 ml	250 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml	100 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
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
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use:

The kit is designed to extract DNA from Urine sample.

Introduction

HiPurA[®] Urine DNA Purification Kit provides a fast and easy method for purification of urine DNA 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 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[®] Urine DNA Purification Kit

This kit simplifies isolation of DNA from urine sample with spin-column procedure. Urine sample is subjected to lysis by Proteinase K in a chaotropic salt solution. Following lysis is the binding of DNA to the silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield high purity DNA. Two rapid wash steps removes trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer (ET) provided with the kit.

HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube] [DBCA03]

HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube] 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 endonuclease-digestion, Southern blotting applications and sequencing reactions.

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® Urine 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

- 0.5M EDTA (pH 8.0)(ML014)
- 70°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® Urine 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 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 to cool to room temperature (15-25°C) before use.
3. Ensure that clean & dry tubes and tips are used for the procedure.

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	8 ml	24 ml
50	20 ml	60 ml
250	100 ml	300 ml

5. Reconstitute Proteinase K (DS2280)

The HiPurA® Urine 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 the Proteinase K is 33.5 units/mg dry weight.

Resuspend the Proteinase K powder in Molecular Biology Grade Water 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.

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 urine sample in a sterile container and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the urine 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: Urine

Sample preparation

Prepare sample by adding 500 µl of 1X PBS to 1ml urine sample. Add 30 µl of 0.5M EDTA (pH 8.0). Vortex thoroughly for 1 minute.

Procedure

1. Add 1 ml of treated urine sample (**see: Sample preparation**) to 2.0 ml collection tube. Centrifuge the urine sample for 2 minutes at 13,000 rpm. Discard the supernatant completely.
2. **Resuspend pellet**
Resuspend the pellet obtained from step 1 with 200 µl of Resuspension Solution (1X PBS) (ML116) and mix thoroughly.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C).

3. **Lyse cells**
Add 20 µl of the Proteinase K solution (20 mg/ml) to the sample, followed by 200 µl of Lysis Solution (C1) (DS0010). Mix thoroughly by vortexing for about 15 seconds and incubate at 70°C for 10 minutes. Continue with step 4 (Binding).

NOTE: A homogeneous mixture is essential for efficient lysis.

4. **Binding**
Add 200 µl of ethanol (96-100%) to the lysate and mix thoroughly by vortexing for 5-10 seconds.

NOTE: A homogeneous solution is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the 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.

5. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
Transfer the lysate obtained from step 4 onto the column provided. Centrifuge at 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. 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 $\geq 14,000$ rpm will not affect the yield or purity of the DNA.

6. **Wash**

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

Add 500 µl of diluted Wash Solution (WS) to the column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

7. Add another 500 µl of diluted Wash Solution (WS) to the column and centrifuge at 13,000 for 3 minutes to dry the column. Centrifuge the 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 200 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 10,000 rpm for 1 minute to elute the DNA.

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

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer, 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.

Storage of the eluate: 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 Purity
Urine sample	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.	Spin column is clogged	Sample is too large	Use smaller quantity of sample, i.e. fewer cells. To salvage the current preparation increase the g- force or spin for longer time until the lysate passes through the binding column to alleviate clogging of column.
		Inefficient disruption of cells	The Proteinase K digestion at 55°C can be extended. Invert the sample tube after Proteinase K digestion for a homogenous mixture. 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.
2.	Low yield of genomic DNA	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. If minimally sheared DNA is required for downstream applications like PCR, mix with gentle pipetting or inversion until homogenous instead of vortexing.
		DNA elution step not performed properly	Ensure that the DNA elution is in 200 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 4 before adding the sample to the HiElute Miniprep Spin Column (Capped) in step 5.
		Eluate contains residual ethanol from the Wash Solution	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, always use a fresh tube for elution.
		Wash Solution Concentrate was not diluted before use	Ensure that the Wash Solution concentrate is properly diluted with ethanol as per preparation 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)
3.	Purity of the DNA is lower than expected; (A_{260}/A_{280} ratio is low).	Eluate was diluted in water for absorbance measurement	Use either the Elution Buffer provided, or 10 mM Tris-HCl, pH 8.0-8.5 as the eluate.
		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.
4.	Purity of the DNA is higher than expected; (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.
5.	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.
		Sample is old, degraded, or has undergone repeated freeze/thaw cycles	Fresh cells should be used immediately. Old material may yield degraded DNA in the eluate. Cells can be frozen in liquid nitrogen and stored at -70°C until needed.
6.	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 (12,000-16,000xg) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The column should be transferred to a new 2.0 ml collection tube before adding the elution buffer.

Please refer disclaimer Overleaf.

Safety Information

HiPura® Urine 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.



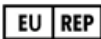








Disposal

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
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

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Disclaimer :

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HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, 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