MB554 HiPurA® Multi-Sample DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB554		
		20 Preps	50 Preps	250 Preps
DS0090	Resuspension Buffer (MS)	5.6 ml	14 ml	70 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0014	Gram- Positive Lysis Solution (GPLA)	6 ml	15 ml	75 ml
DS0011	Prewash Solution Concentrate (PW)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 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
MB098	Lysozyme	0.27 g	0.675 g	3.375 gm
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/ animal blood, cells, tissue, gram positive and gram negative bacterial samples.

Introduction

HiPurA® Multi-Sample DNA Purification Kit provides a fast and easy method for purification of total DNA from a wide variety of samples 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® Multi-Sample DNA Purification Kit

This kit simplifies isolation of DNA from fresh, old (more than 24 hours) and frozen blood, bacteria (Gram-positive and Gram-negative), tissues and cells with spin-column procedure.

Genomic DNA purification from blood involves cell lysis by incubating the whole blood in a solution containing chaotropic ions in the presence of Proteinase K at 55°C. After the initial binding of DNA onto the HiElute Miniprep Spin Column (Capped), impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing

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

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

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.

For genomic DNA isolation from bacteria, cells are grown in a medium till they reach log phase and are harvested by centrifugation. After harvesting, the bacterial (Gram-positive) cell wall is degraded by lysozyme and Proteinase K. For Gram-negative bacteria, the lysozyme treatment is not required. Following lysis, the DNA is bound 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 remove trace amount of salt and protein contaminants resulting in the elution of pure DNA in the Elution Buffer provided with the kit.

Genomic DNA purification from animal tissue (spliced and digested), suspended/attached animal cells, digested rodent tail tissue is done by subjecting the samples 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 purified DNA. 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. Typical DNA yield from 1 x 10^6 cells is 10-20 μg and 30 mg of tissue yields 30-45 μg of DNA.

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. 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.

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® Multi-Sample 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

- 55°C water bath or heating block (For bacteria, tissues, rodent tails, blood)
- 37°C water bath or heating block (For Gram-positive bacteria only)
- 70°C water bath or shaking water bath (For cultured cells and tissues)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Molecular Biology Grade Water (Product code: ML024)
- Trypsin (Product code: TCL034)

Storage

Store the HiPurA® Multi-Sample 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 55°C.
   (For Gram-positive, Gram-negative bacteria, blood, tissues and rodent tail)

2. Preheat a water bath or heating block to 37°C.
   (For Gram-positive bacteria only)

3. Preheat a water bath or heating block to 70°C.
   (For cultured cells and tissues)

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. The reagent should be at room temperature (15-25°C) before use.

5. Ensure that clean & dry tubes and tips are used for the procedure.

6. **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

7. **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

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

   The HiPurA® Multi-Sample 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 weights.
   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.

9. **Prepare Lysozyme Solution (Product Code: MB098) For Gram-positive bacteria only**

Prepare a 45 mg/ml solution of Lysozyme (approximately 2.115 x 10^6 unit/ml) with the Gram-Positive Lysis Solution (GPLA) as the diluent, which is provided in the kit. Lysozyme solution should be freshly prepared prior to use.
Example: In order to make 1 ml of Lysozyme solution, dissolve 45 mg of lysozyme (provided) in 1 ml of Gram-Positive Lysis Solution (GPLA). Mix by pipetting or vortex to dissolve the lysozyme.

NOTE: Lysozyme dissolves readily by pipetting as opposed to vortexing. Excessive vortexing may cause foaming.

For each DNA preparation, 200 µl of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at -20°C.

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 human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. 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 tissue is at room temperature before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: human tissue, blood, cells
Animal samples: blood, cells

Procedure

I. Lysis

1. Blood

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

NOTE: We recommend freezing blood in 200 µl aliquots. 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 Buffer (MS) to bring the volume upto 200 µl.

i. Add 20 µl of the reconstituted Proteinase K solution (20 mg/ml) **(Refer to General Instructions Preparation)** 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).

ii. **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 and continue with step II (Binding).

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

2. Cultured Cell

i. **Harvest cells**

- **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto 5 x 10^6 cells for 5 minutes at 300 x g (≈1500 rpm). Discard the culture medium and continue with step ii of cultured cell.
- **Suspension cell cultures:** Centrifuge upto 5 x 10^6 cells for 5 minutes at 300 x g [≈1500 rpm]. Discard the culture medium completely and continue with step ii of cultured cell.

NOTE: Cells can be harvested, by aliquoting in 2.0 ml microcentrifuge tubes and flash-freezing in liquid nitrogen, these can be stored at -70°C for several months before preparing DNA.

ii. **Resuspend cells**

Resuspend the pellet obtained from step i of cultured cell, in 2ml capped collection tube 200 µl of Resuspension Buffer (MS) (DS0090) and mix thoroughly. If previously frozen, allow the cell pellet to thaw slightly before resuspending. If residual RNA is not a concern, continue with step iii of cultured cell.

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), then continue with step iii of cultured cell.

iii. **Lyse cells**

Add 20 µl of the Proteinase K solution (20 mg/ml) **(Refer to General Instruction Preparation)** to the sample from step i of cultured cell, 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.

NOTE: A homogeneous mixture is essential for efficient lysis. Continue with step II (Binding).

C. <u>Mammalian Tissue</u>

i. **Prepare tissue**

Weigh a piece of fresh or frozen tissue and mince quickly. If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation. Cut the tissue into small pieces as it enables more efficient lysis. Up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a given capped 2.0 ml collection tube and continue to step 2 of Mammalian Tissue Preparation.

NOTE: If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation.

NOTE: Tissue can be harvested, by aliquoting in capped 2.0 ml collection tubes and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing DNA.

ii. **Digest tissue**

Add 180 µl of Resuspension Buffer (MS) (DS0090) and 20 µl of the Proteinase K solution (20 mg/ml) **(Refer to General Instruction Preparation)** to the tissue. Mix by vortexing. Incubate the sample at 55°C until the tissue is completely digested with no particles remaining. Mix by vortexing occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. Vortex briefly after digestion is completed.

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), then continue with step iii of Mammalian tissue.

iii **Lyse cells**

Add 200µl of Lysis Solution (C1) (DS0010) to the sample. Mix by vortexing thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes. Continue with step II (Binding).

D. Rodent Tail

i. **Prepare rodent tails**

Measure and cut a piece of fresh or frozen rodent tail. Before cutting, allow the frozen rodent tail to thaw slightly keep on ice in order to protect degradation. Do not use more than 0.6 cm (rat) or 1.2 cm (mouse) tail per preparation. Cut two (mouse) or one (rat) 0.5-0.6 cm lengths of tail and place them in a capped 2.0 ml collection tube. Continue with step ii.

NOTE: Rodent tails can be stored at -20°C for several months before preparing DNA.

ii. **Digest tissue**

Add 180 µl of Resuspension Buffer (MS) (DS0090) and 20 µl of the Proteinase K solution (20 mg/ml) **(Refer to General Instruction Preparation)** to the rodent tail. Mix by vortexing. Ensure that the tail is fully submerged. Incubate the sample at 55°C until the tail tissue is completely digested. Some particles (bone and hair) may remain. Mix by vortexing occasionally or use a shaking water bath, during incubation for more rapid digestion. Digestion is usually complete in 3 to 6 hours. Vortex briefly after digestion is complete. If residual RNA is not a concern, continue with step iii Rodent tail.

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), then continue with step iii of Rodent tail.

iii. **Lysis reaction**

Add 200 µl of Lysis Solution (C1) (DS0010) to the sample. Vortex thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes. Continue with step II (Binding).

E. Gram-Negative Bacteria

i. **Harvesting of cells**

Pellet 1.5 ml of bacterial broth culture in 2.0ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g (≈13,000-16,000 rpm). Remove the culture medium completely and discard.

NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column (Capped).

ii. **Resuspend cells**

Resuspend the pellet thoroughly in 180µl Resuspension Buffer (MS) (DS0090).

i. **Prepare for cell lysis**

Add 20 µl of the Proteinase K solution (20mg/ml) **(Refer to General Instruction Preparation)** to the sample. Mix and incubate for 30 minutes at 55°C. If residual RNA is not a concern, continue with step iv of gram negative bacteria.

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 iv of gram negative bacteria.

ii. **Lyse cells**
Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds) and incubate at 55°C for 10 minutes. Continue with step II (Binding).

NOTE: A homogeneous mixture is essential for efficient lysis.

F. <u>Gram-Positive Bacteria</u>

i. Prepare Lysozyme Solution using Lysozyme from chicken egg white, which is provided in the kit.
Prepare a 45 mg/ml stock solution of lysozyme as described under General Preparation Instructions. 200 µl of Lysozyme Solution is required per isolation procedure. Prepare extra solution to account for pipetting error.

NOTE: (Optional) For higher yields - If working with *Staphylococcus* species, supplement the Lysozyme Solution with 200 units/ml of lysostaphin (not provided). For *Streptococcus* species, supplement the Lysozyme Solution with 250 units/ml of mutanolysin (not provided).

ii. **Harvest Cells**
Pellet 1.5 ml of bacterial broth culture in 2.0ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g (≈13,000-16,000rpm). Remove the culture medium completely and discard.

NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiElute Miniprep Spin Column (Capped).

iii. **Resuspend cells**
Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step i of gram positive bacteria) and incubate for 30 minutes at 37°C.

iv. **Lyse cells**
Add 20µl of the Proteinase K solution (20 mg/ml) **(Refer to General Instruction Preparation)** to the sample. If residual RNA is not a concern continue with step v of gram positive bacteria.

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 v of gram positive bacteria.

v. Add 200 µl of Lysis Solution (C1) (DS0010). Vortex thoroughly for few seconds and incubate at 55°C for 10 minutes, then continue with step II (Prepare for binding).

NOTE: A homogeneous mixture is essential for efficient lysis.

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

NOTE: A homogenous solution is essential.

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

Transfer the lysate obtained from step II onto the HiElute Miniprep Spin Column (Capped) (in DBCA016 Collection Tube) provided. Centrifuge at ≥6,500 x g (≈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.

IV. Prewash

(Prepare Prewash Solution Concentrate (PW) as indicated in General Preparation Instructions)

Add 500 µl of diluted Prewash Solution (PW) (DS0011) to the column 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.

V. Wash

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

Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at 12,000 – 16,000 x g (≈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. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped 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.

VI. 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 ≥6,500 x g (≈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; however, the overall 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 200µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

VII. 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
Human blood	4-12 µg	1.6-1.9
CHO cells	10-45 µg	1.6-1.9

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 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	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). 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 minute 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.
		Wash Solution Concentrate was not diluted before use	Ensure that the Wash Solution concentrate is properly diluted with ethanol as per general preparation instructions.
3.	Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is less)	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	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, tissues, rodent tail, whole blood should be used immediately. Old material may yield degraded DNA in the eluate. Cells and tissues can be frozen in liquid nitrogen and stored at -70°C until needed. Rodent tails can be stored at -20°C for several weeks or -70°C for several months. Whole blood can be stored at 4ºC for up to 12 hours.
		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.
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 (12,000-16,000 x g) 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 wash solution.

Safety Information

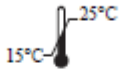
The HiPurA® Multi-Sample DNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing 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
	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
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
	02/2025

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