## Intended Use

Recommended for isolation of DNA from human blood samples.

## Introduction

HiPurA® 96 Blood Genomic DNA Purification Kit provides 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 96-well format comprises of three steps viz. adsorption of DNA to the silica gel membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia’s 96-well format allows rapid processing of multiple samples. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, ligation, PCR and sequencing.

### HiPurA® 96 Blood Genomic DNA Purification Kit

This kit simplifies isolation of DNA from fresh and old (more than 24 hours) blood. 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 70°C. HiMedia’s HiPurA® 96-well DNA Plate 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.

### Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents provided</th>
<th>MB521</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 X 96 Preps</td>
</tr>
<tr>
<td>ML116</td>
<td>Resuspension Solution (1X PBS)</td>
<td>30 ml</td>
</tr>
<tr>
<td>DS0010</td>
<td>Lysis Solution (C1)</td>
<td>30 ml</td>
</tr>
<tr>
<td>DS0011</td>
<td>Prewash Solution Concentrate (PW)</td>
<td>24 ml</td>
</tr>
<tr>
<td>DS0012</td>
<td>Wash Solution Concentrate (WS)</td>
<td>30 ml</td>
</tr>
<tr>
<td>DS0040</td>
<td>Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]</td>
<td>30 ml</td>
</tr>
<tr>
<td>MB086</td>
<td>Proteinase K</td>
<td>44 mg</td>
</tr>
<tr>
<td>DS0003</td>
<td>RNase A Solution (20 mg/ml)</td>
<td>3 ml</td>
</tr>
<tr>
<td>LWB-16-96</td>
<td>HiPurA® 96-well Block (1.6 ml)</td>
<td>4 nos</td>
</tr>
<tr>
<td>DBPL-96-01</td>
<td>HiPurA® 96-well DNA Plate</td>
<td>1 no</td>
</tr>
<tr>
<td>DVB-96</td>
<td>HiPurA® 96-well V-Block</td>
<td>1 no</td>
</tr>
<tr>
<td>PR11</td>
<td>HiPurA® Silicon Pad for sealing</td>
<td>1 no</td>
</tr>
</tbody>
</table>
HiPurA® 96-well DNA Plate (DBPL-96-01)

HiPurA® 96-well DNA Plate is based on the advanced silica binding principle presented in a vacuum format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica membrane to yield high quantity of DNA.

It facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. It eliminates the need for alcohol precipitation, expensive resins, and harmful 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 during wash steps, leaving pure nucleic acids to be eluted in the buffer provided with the kit. Blood genomic DNA up to 20-30 kb in length can be purified for further downstream applications.

Elution

An elution with 100 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 100 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size up to 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® 96 Blood 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

- 70°C incubator or oven
- Tabletop Centrifuge with swinging bucket rotor and plate carriers (5,000 rpm required at least)
- Vacuum Manifold for 96 well plate
- Vacuum source (capable of –25-30 inches Hg)
- Vacuum regulator
- Multi-channel pipette with tips
- Ethanol (96 – 100%)
- Molecular biology grade water (Product code: ML024)

Storage

Store the HiPurA® 96 Blood Genomic DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition, the kit is stable for 1 year.
General Preparation Instructions

1. Preset the temperature of the incubator or oven 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. Dilute Prewash Solution Concentrate (PW) (DS0011) as follows:

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Prewash Solution Concentrate (PW)</th>
<th>Ethanol (96-100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 X 96 Preps</td>
<td>24 ml</td>
<td>36 ml</td>
</tr>
<tr>
<td>4 X 96 Preps</td>
<td>96 ml</td>
<td>144 ml</td>
</tr>
<tr>
<td>12 X 96 Preps</td>
<td>288 ml</td>
<td>432 ml</td>
</tr>
</tbody>
</table>

NOTE: User should carry out dilution of Wash Solution Concentrate in a separate container, as it is not provided with this kit.

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

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Wash Solution Concentrate (WS)</th>
<th>Ethanol (96-100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 X 96 Preps</td>
<td>30 ml</td>
<td>90 ml</td>
</tr>
<tr>
<td>4 X 96 Preps</td>
<td>120 ml</td>
<td>360 ml</td>
</tr>
<tr>
<td>12 X 96 Preps</td>
<td>360 ml</td>
<td>1.08 lit</td>
</tr>
</tbody>
</table>

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.

5. Reconstitute Proteinase K (MB086)
   The HiPurA® 96 Blood Genomic DNA Puri fication 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 powder in molecular biology grade water to obtain a 20 mg/ml stock solution.

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Proteinase K</th>
<th>Molecular Biology Grade Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 X 96 Preps</td>
<td>44 mg</td>
<td>2.2 ml</td>
</tr>
<tr>
<td>4 X 96 Preps</td>
<td>176 mg</td>
<td>8.8 ml</td>
</tr>
<tr>
<td>12 X 96 Preps</td>
<td>528 mg</td>
<td>26.4 ml</td>
</tr>
</tbody>
</table>

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 centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10. The 96-well accessories 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

**General Note:**

1. If less than 96 samples are processed at a time, seal the unused wells properly with the provided HiPurA® Silicon Pad for sealing (PR11).
2. The HiPurA® Silicon Pad for sealing (PR11) used in the protocol should not be discarded. It can be reused after wiping with ethanol and washing properly.

**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

**For Frozen blood:** Frozen blood should be equilibrated to Room Temperature (15-25°C) before beginning the procedure.

**NOTE:** DO NOT FREEZE AND THAW THE BLOOD FOR MORE THAN ONE TIME. It may affect the yield and integrity of DNA.

**NOTE:** If the sample is less than 200 µl, add the Resuspension solution (ML116) to bring the volume up to 200 µl. Proceed with the Procedure for DNA Extraction.

**Sample Storage:**

Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results.

For short-term storage of up to 10 days, collect blood in tubes containing EDTA as and anticoagulant, and store at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at -70°C.
Procedure for DNA Extraction

Centrifugation Based Protocol

1. Add 20 µl of the Proteinase K solution (20mg/ml) (Refer to General Preparation Instructions) into each well of the HiPurA® 96-well Block (1.6 ml) (LWB-16-96).

2. Add 200 µl of the whole blood to each well of the block.

   NOTE: If the sample is less than 200 µl, add the Resuspension Solution (ML116) to bring the volume up to 200 µl. Whole blood may be stored at 4°C for at least 3 months before preparing the DNA. If residual RNA is not a concern, continue with step 4.

   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 4.

3. Lysis reaction
   Add 200 µl of the Lysis Solution (C1) (DS0010) to each sample, taking care not to wet the rims of the wells. Seal the wells using a HiPurA® Silicon Pad for sealing (PR11). Ensure that the wells are properly sealed to avoid leaks during shaking.

4. Mix thoroughly by shaking vigorously for 30 seconds.

   NOTE: For efficient lysis, it is essential that the samples and Lysis Solution (C1) are mixed immediately and thoroughly to yield a homogeneous solution. Hold the sealed HiPurA® 96-well Block with both hands and shake up and down vigorously.

5. Incubate at 70°C for 10 minutes in an incubator or oven.

6. 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.

7. Load lysate in HiPurA® 96-well DNA Plate (DBPL-96-01)
   Place the HiPurA® 96-well DNA Plate (DBPL-96-01) onto a new HiPurA® 96-well Block (1.6 ml) (LWB-16-96). Carefully transfer the lysate from HiPurA® 96-well Block (1.6ml) from step 6 to the corresponding wells in HiPurA® 96-well DNA Plate (DBPL-96-01). Place the HiPurA® Silicon Pad for sealing (PR11) over it. Centrifuge the HiPurA® 96-well DNA Plate at 5,000 rpm for 5 minutes.

   Discard the flow-through liquid from HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and reuse the block.

8. Prewash
   (Prepare Prewash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Prewash Solution to each well of HiPurA® 96-well DNA Plate (DBPL-96-01) and place the HiPurA® Silicon Pad for sealing (PR11) over it. Centrifuge the HiPurA® 96-well DNA Plate at 5,000 rpm for 5 minutes. Discard the flow-through liquid and reuse the HiPurA® 96-well Block (1.6 ml).
9. **Wash**
*(Prepare Wash Solution as indicated in General Preparation instructions)*
Add 500 µl of diluted Wash Solution (WS) (DS0012) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01) and place the HiPurA® Silicon Pad for sealing (PR11) over it. Centrifuge the HiPurA® 96-well DNA Plate at 5,000 rpm for 5 minutes. Discard the flow-through liquid and reuse the HiPurA® 96-well Block (1.6 ml). Repeat this wash step one more time.

10. Discard the flow-through liquid, place the HiPurA® 96-well DNA Plate back onto the same HiPurA® 96-well Block (1.6 ml). Place the HiPurA® Silicon Pad for sealing (PR11) over it and centrifuge for 10 minutes at 5,000 rpm to remove the traces of ethanol present in the Wash Solution.

11. Discard any flow-through and incubate the HiPurA® 96-well DNA Plate open without the HiPurA® Silicon Pad, for 15 minutes at 70°C in an incubator to evaporate residual ethanol.

12. **DNA Elution**
Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96), which was used as collection tray, and replace it with a HiPurA®™ 96-well V-Block for elution. Place the HiPurA® 96-well DNA Plate (DBPL-96-01) on to it. Add 100 µl of Elution Buffer (ET) (DS0040) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature for 5 minutes. Centrifuge at approximately 5,000 rpm for 5 minutes.

**NOTE:** Elution can be repeated with another 100 µl of Elution Buffer (ET); however, the DNA concentration will be low.

13. Remove the HiPurA®™ 96-well V-Block, which contains eluted genomic DNA samples.

**Vacuum Based Protocol**
1. Add 20 µl of the Proteinase K solution (20 mg/ml) *(Refer to General Preparation Instructions)* into each well of the HiPurA® 96-well Block (1.6 ml) (LWB-16-96).

2. Add 200 µl of the whole blood to each well of the HiPurA® 96-well Block (1.6 ml).

**NOTE:** If the sample is less than 200 µl, add the Resuspension Solution (ML116) to bring the volume up to 200 µl. Whole blood may be stored at 4°C for at least 3 months before preparing the DNA. If residual RNA is not a concern, continue with step 4.

**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 4.

3. **Lysis reaction**
Add 200 µl of the Lysis Solution (C1) (DS0010) to each sample, taking care not to wet the rims of the wells. Seal the wells using a HiPurA® Silicon Pad for sealing (PR11). Ensure that the wells are properly sealed to avoid leaks during shaking.

4. Mix thoroughly by shaking vigorously for 30 seconds.

**NOTE:** For efficient lysis, it is essential that the samples and Lysis Solution (C1) are mixed immediately and thoroughly to yield a homogeneous solution. Hold the sealed HiPurA® 96-well Block with both hands and shake up and down vigorously.

5. Incubate at 70°C for 10 minutes in an incubator or oven.
6. **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.

7. **Load lysate in HiPurA® 96-well DNA Plate (DBPL-96-01)**

Connect the vacuum manifold to the vacuum source. Remove the manifold top and place a new HiPurA® 96-well Block (1.6 ml) (LWB-16-96) into the manifold base to collect the flow-through liquid. Replace the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) onto the manifold top.

8. Carefully transfer the lysate from HiPurA® 96-well Block (1.6 ml) from step 6 to the corresponding wells in HiPurA® 96-well DNA Plate (DBPL-96-01). Place the HiPurA® Silicon Pad for sealing (PR11) over it.

**NOTE:** Any Vacuum Manifold, which can accommodate 96-well plates, can be used with the HiPurA® 96-well DNA Plate (DBPL-96-01). A negative pressure of –25-30 inches Hg is required. We recommend the use of a vacuum regulator to adjust the negative pressure. –25-30 inches Hg is equivalent to approximately –850-1000 mbar and –12 to 15 psi.

9. Turn on the vacuum source and adjust it to –25-30 inches Hg. Continue to draw vacuum through the plates until no liquid remains in any of the wells of the HiPurA® 96-well DNA Plate.

10. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the manifold and temporarily set it aside on a piece of absorbent towel or plastic wrap. Discard the flow-through liquid from HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and reuse the block.

11. **Prewash**

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

Add 500 µl of diluted Prewash Solution to each well of HiPurA® 96-well DNA Plate (DBPL-96-01) and place the HiPurA® Silicon Pad for sealing (PR11) over it. Continue to draw vacuum until no Prewash Solution is present in any of the wells. Discard the flow-through liquid and reuse the HiPurA® 96-well Block (1.6 ml).

12. **Wash**

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

Add 500 µl of diluted Wash Solution (WS) (DS0012) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01). Apply vacuum until no Wash Solution is present in any of the wells. Discard the flow-through liquid and reuse the HiPurA® 96-well Block (1.6 ml). Repeat this wash step one more time.

13. Discard the flow-through liquid, place the HiPurA® 96-well DNA Plate onto a new HiPurA® 96-well Block (1.6 ml). Place the HiPurA® Silicon Pad for sealing (PR11) over it and apply vacuum at a maximum of –30 inches Hg for 10 minutes to remove the traces of ethanol present in the Wash Solution.

14. Turn off the vacuum source. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the vacuum manifold and vigorously tap the plate approximately 6-8 times on
several layers of absorbent toweling. Be careful not to damage the drip directors on the underside of the plate.

**NOTE:** Lint-free absorbent toweling is recommended to avoid the release of tiny fibres, which could contaminate the plasmid and interfere with subsequent downstream applications.

15. Incubate HiPurA® 96-well DNA Plate open without the HiPurA® Silicon Pad for 15 minutes at 70°C in a vacuum oven to evaporate residual ethanol.

**NOTE:** If vacuum oven is not available then connect the vacuum manifold to the oven pre-heated at 70°C through the port provided on the oven.

16. **DNA Elution**

Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96) which was used as collection tray from the vacuum manifold base and replace it with a new HiPurA® 96-well V-plate for elution. Reassemble the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) on the HiPurA® 96-well V-plate. Add 100 µl of Elution Buffer (ET) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature for 5 minutes. Turn on the vacuum source and allow the vacuum (-30 Hg pressure) to continue for 10 minutes.

**NOTE:** Elution can be repeated with another 100 µl of Elution Buffer (ET); however, the DNA concentration will be low.

17. Turn off the vacuum source and use the vacuum regulator to gradually release the vacuum from the manifold. Disassemble the manifold and remove the HiPurA® 96-well V-plate, which contains eluted genomic DNA samples.

**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**

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>DNA Yield</th>
<th>DNA Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 µl whole blood</td>
<td>4-12 µg/well</td>
<td>1.6-1.9</td>
</tr>
</tbody>
</table>
References:

Trouble shooting Guide:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Presence of cell clumps/colored residue on the 96-well membrane after washing.</td>
<td>Inefficient cell lysis due to improper mixing of the lysis buffer with the blood sample</td>
<td>The sample and the Lysis Solution (C1) should be mixed thoroughly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Due to decreased Proteinase K activity</td>
<td>Do not add Proteinase K directly to the Lysis Solution (C1). Ensure that the stock solution is stored as indicated.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prewash (PW) and Wash Solution (WS) prepared incorrectly</td>
<td>Ensure that Prewash (PW) and Wash Solution (WS) concentrates are diluted with correct volumes of ethanol.</td>
</tr>
<tr>
<td>2.</td>
<td>Poor or low genomic DNA recovery</td>
<td>Sample is old or degraded</td>
<td>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 future use, for at least 3 months).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNA elution is improper</td>
<td>To improve the DNA yield, incubate for 5 minutes at room temperature after it is added to the 96-well membrane.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eluate contains residual ethanol from wash steps</td>
<td>Remove ethanol from the second wash completely before eluting the DNA as mentioned in the procedure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use of water instead of Elution Buffer for elution of DNA.</td>
<td>Elution Buffer (ET) is recommended for optimal yields and storage of 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.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NOTE:</strong> Only DNase/RNase and Protease free water should be used for eluting DNA</td>
</tr>
<tr>
<td>3.</td>
<td>Purity of the DNA is lower than expected</td>
<td>Eluate was diluted in water for absorbance measurement.</td>
<td>Use the Elution Buffer provided.</td>
</tr>
</tbody>
</table>
(A260/A280 ratio is less)  Purification is incomplete due to well overloading or inadequate lysis.  Reduce the initial volume of the sample or increase the lysis time while monitoring the lysis visually.

4. A260/A280 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 until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

Safety Information
The HiPurA® 96 Blood 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.