HiPurA® Mammalian Genomic DNA Purification Kit

Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents provided</th>
<th>MB506</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML116</td>
<td>Resuspension Solution (1X PBS)</td>
<td>6 ml 15 ml 75 ml</td>
</tr>
<tr>
<td>DS0010</td>
<td>Lysis Solution (C1)</td>
<td>6 ml 15 ml 75 ml</td>
</tr>
<tr>
<td>DS0015</td>
<td>Lysis Solution (AL)</td>
<td>5.6 ml 14 ml 70 ml</td>
</tr>
<tr>
<td>DS0012</td>
<td>Wash Solution Concentrate (WS)</td>
<td>8 ml 20 ml 100 ml</td>
</tr>
<tr>
<td>DS0040</td>
<td>Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]</td>
<td>6 ml 15 ml 75 ml</td>
</tr>
<tr>
<td>MB086</td>
<td>Proteinase K</td>
<td>10 mg 25 mg 125 mg</td>
</tr>
<tr>
<td>DS0003</td>
<td>RNase A Solution (20 mg/ml)</td>
<td>0.5 ml 1.25 ml 6.25 ml</td>
</tr>
<tr>
<td>DBCA03</td>
<td>HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]</td>
<td>20 nos 50 nos 250 nos</td>
</tr>
<tr>
<td>DBCA016</td>
<td>Collection Tube (Uncapped), Polypropylene (2.0 ml)</td>
<td>20 nos 50 nos 250 nos</td>
</tr>
<tr>
<td>PW1139</td>
<td>Collection Tube, Polypropylene (2.0 ml)</td>
<td>40 nos 100 nos 2 X 250 nos</td>
</tr>
</tbody>
</table>

Intended Use

Recommended for isolation of DNA from cells, blood and tissue samples of human and animal origin.

Introduction

HiPurA® Mammalian Genomic DNA Purification Kit provides a fast and easy method for purification of total 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® Mammalian Genomic DNA Purification Kit

This kit simplifies isolation of DNA from tissues or cells with spin-column procedure. Human/Animal tissue (spliced and digested), suspended/attached human/animal cells, whole blood or digested rodent tail tissue 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. Typical DNA yield from 1 x 10⁶ 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 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 \( \frac{A_{260} - A_{320}}{A_{280} - A_{320}} \) 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® Mammalian 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

- 55°C water bath or shaking water bath
- 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® Mammalian 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. **Preheat a water bath or shaking water bath to 55°C**  
   (For use with tissues, rodent tails, fresh whole blood and white blood cells).

2. **Preheat a water bath or heating block to 70°C**  
   (For use with cultured cells and tissues)
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 to cool to room temperature (15-25°C) before use.

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

5. **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>20</td>
<td>8 ml</td>
<td>24 ml</td>
</tr>
<tr>
<td>50</td>
<td>20 ml</td>
<td>60 ml</td>
</tr>
<tr>
<td>250</td>
<td>100 ml</td>
<td>300 ml</td>
</tr>
</tbody>
</table>

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

The HiPurA® Mammalian 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 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.

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Proteinase K</th>
<th>Molecular Biology Grade Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10 mg</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>50</td>
<td>25 mg</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>250</td>
<td>125 mg</td>
<td>6.25 ml</td>
</tr>
</tbody>
</table>

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 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. Ensure that the tissue is at room temperature before beginning the protocol.

Types of Specimen

Clinical samples: human tissue
Animal tissue: liver, lungs, muscle

Procedure

A. Cultured Cell Preparation

1. Harvest cells
   - **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto $5 \times 10^6$ cells for 5 minutes at $300 \times g \approx 1500$ rpm. Discard the culture medium and continue with step 2 of Cultured Cell Preparation.
   - **Suspension cell cultures:** Centrifuge upto $5 \times 10^6$ cells for 5 minutes at $300 \times g \approx 1500$ rpm. Discard the culture medium completely and continue with step 2 of Cultured Cell Preparation.

   **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.

2. Resuspend cells
   Resuspend the pellet obtained from step 1 of Cultured Cell Preparation, in capped 2ml centrifuge tube add 200 µl of Resuspension Solution (1X PBS) (ML116) 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 3 of Cultured Cell Preparation.

   **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 3a.

3. Lyse cells
   Add 20 µl of the Proteinase K solution (20 mg/ml) to the sample from step 2 of Cultured Cell Preparation, 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 F (Binding).

B. Mammalian Tissue Preparation

1. 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:** Tissue can be harvested, by aliquoting in 2.0 ml collection tubes and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing DNA.
2. **Digest tissue**  
   Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution (20 mg/ml) 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 3 of Mammalian Tissue Preparation.

3. **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 F (Binding).

C. **Rodent Tail Preparation**

1. **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 2.0 ml capped collection tube. Continue with step 2 of Rodent Tail Preparation.

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

2. **Digest tissue**  
   Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution (20 mg/ml) 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 3 of Rodent Tail Preparation.

   **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 3 of Rodent Tail Preparation.

3. **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 F (Binding).

D. **Whole blood Preparation**

1. **Collect blood**  
   Collect whole blood in an anticoagulant tube (an EDTA tube is preferred). Whole blood should be equilibrated to room temperature (15-25°C) before beginning the preparation.
2. **Prepare blood**  
   Add 20 µl of the Proteinase K solution (20 mg/ml) into a capped 2.0 ml collection tube containing 200 µl of the whole blood. If the sample is less than 200 µl, add the Resuspension Solution (1X PBS) (ML116) to bring the volume up to 200 µl.  
   
   **NOTE:** If the sample is already dispensed into a tube, the Proteinase K solution can be added to the sample. Vortex to ensure thorough mixing of the enzyme. 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 3 of Whole blood Preparation.  
   
   **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 3 of Whole blood Preparation.  
   
3. **Lyse cells**  
   Add 200 µl of Lysis Solution (C1) (DS0010) to the sample. Vortex thoroughly for 15 seconds. A homogeneous mixture is essential for efficient lysis. Incubate at 55°C for 10 minutes. Continue with step F (Binding).  
   
**E. White Blood Cell (WBC) Preparation**  
   
1. **Prepare white blood cells from whole blood**  
   Prepare WBCs from 500 µl of whole blood per preparation, see appendix for ammonium chloride lysis procedure.  
   
2. **Resuspend cells**  
   In a capped 2ml collection tube resuspend the pellet thoroughly in 200 µl of the Resuspension Solution (1X PBS) (ML116) and add 20 µl of the Proteinase K solution (20 mg/ml) to the sample. Vortex briefly to ensure thorough mixing of the enzyme. If residual RNA is not a concern, continue with step 3 of White Blood Cell (WBC) Preparation.  
   
   **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 3 of White Blood Cell (WBC) Preparation.  
   
3. **Lyse cells**  
   Add 200 µl of Lysis Solution (C1) (DS0010) to the sample. Vortex thoroughly. A homogeneous mixture is essential for efficient lysis. Incubate at 55°C for 10 minutes. Continue with step F (Binding).  
   
**DNA Isolation From All Listed Sample Types**  
This is a continuation of the procedure for DNA purification from the samples prepared in Sections A, B, C, D and E.  
   
**F. 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.
G. Load lysate in HiElute Miniprep Spin Column (Capped)

Transfer the lysate obtained from step F onto the column provided. Centrifuge at $\geq 6,500 \times g$ ($\approx 10,000$ 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.

H. Wash
(Prepare Wash Solution as indicated in General Preparation Instructions)

Add $500 \mu l$ of diluted Wash Solution to the column and centrifuge at $\geq 6,500 \times g$ ($\approx 10,000$ rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

I. Add another $500 \mu l$ of diluted Wash Solution to the column and centrifuge at $12,000 - 16,000 \times g$ ($\approx 13,000-16,000$ rpm) 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.

J. DNA Elution

Pipette $200 \mu l$ 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 $\geq 6,500 \times g$ ($\geq 10,000$ rpm) for 1 minute to elute the DNA.

**Optional:** A second elution can be collected by repeating step J.

K. 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 $200 \mu l$ increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

**Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-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>WBC’s</td>
<td>Upto 30-45 μg</td>
<td>1.6-1.9</td>
</tr>
<tr>
<td>CHO cell lines</td>
<td>Upto 30-45 μg</td>
<td>1.6-1.9</td>
</tr>
</tbody>
</table>

References:

Troubleshooting 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>Spin column is clogged</td>
<td>Sample is too large or improperly trypsinized</td>
<td>Use smaller quantity of sample, i.e. fewer cells, smaller pieces of tissue etc. 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 tissues or rodent tail</td>
</tr>
<tr>
<td>2.</td>
<td>Low yield of genomic DNA</td>
<td>Lysate/ethanol mixture is not homogenous</td>
<td>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</td>
</tr>
<tr>
<td>Issue</td>
<td>Resolution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol was omitted during binding</td>
<td>Ensure that ethanol is added in step F before adding the sample to the HiElute Miniprep Spin Column (Capped) in step G.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eluate contains residual ethanol from the Wash Solution</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash Solution Concentrate was not diluted before use</td>
<td>Ensure that the Wash Solution concentrate is properly diluted with ethanol as per preparation instructions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of water instead of Elution Buffer for elution of DNA</td>
<td>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. <em>(NOTE: Only DNase/RNase and Protease free water should be used for eluting DNA)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Purity of the DNA is lower than expected; <em>(A$<em>{260}$/A$</em>{280}$ ratio is low).</em></td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background reading is high due to silica fines</td>
<td>Spin the DNA sample at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Purity of the DNA is higher than expected; <em>(A$<em>{260}$/A$</em>{280}$ ratio is too high)</em></td>
<td>RNA contamination                                                            RNase A treatment can be included in future isolations or the final product can be treated with RNase A Solution and repurified.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Shearing of genomic DNA</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 4°C for up to 12 hours.

| 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.

**Appendix: Ammonium chloride lysis procedure for isolating white blood cells from whole blood.**

1) Collect whole blood in an anticoagulant tube.

2) Add 500 µl of whole blood to 1 ml of ammonium chloride lysis solution*. Gently mix (by inversion or on a table rocker) at room temperature for 5 minutes and spin for 5 minutes at 700 x g (≈3200 rpm) in a collection tube.

3) Discard the supernatant. Gently resuspend pellet in 1 ml of ammonium chloride lysis solution; mix gently and spin for 5 minutes at 700 x g (≈ 3200 rpm) in a microcentrifuge. Discard the supernatant, keep the cell pellet on ice and continue with White Blood Cell (WBC) Preparation.

*Ammonium chloride Lysis Solution - 160 mM Ammonium chloride (Product code: MB054) - 20 mM Sodium bicarbonate (Product code: MB045) (Adjust pH to 7.2 with HCl)

**Safety Information**

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

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.
Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.

Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.