HiPer® Blood Genomic DNA Extraction Teaching Kit (Column Based)

Product Code: HTBM006

Number of experiments that can be performed: 10/20

Duration of Experiment
Protocol: 1 hour
Agarose Gel Electrophoresis: 1 hour

Storage Instructions:
- The kit is stable for 6 months from the date of manufacture
- Store Control DNA and Proteinase K at -20°C
- Store 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)
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Aim:

Isolation and purification of genomic DNA from whole blood (using spin column).

Introduction:

Blood is a specialized body fluid composed of cells suspended in a liquid called blood plasma. Whole blood contains three types of cells:

1. Red blood cells (RBCs)
2. White blood cells (WBCs)
3. Platelets

Red blood cells (RBCs) do not have any DNA, as they lose their nuclei during maturation. The white blood cell (WBC) component of the blood contains DNA. The blood sample is treated with detergents which break open the cell membrane to release the contents. Enzymes are then used to break down all the proteins, RNA, sugars and fats in the solution.

Blood Genomic DNA Extraction teaching Kit (Column Based) is designed for rapid extraction and purification of pure genomic DNA from whole blood. It has many advantages over the crude methods of DNA extraction and does not contain harmful organic compounds such as phenol and chloroform.

The DNA purification procedure using the miniprep spin columns comprises of three steps:

- Adsorption of DNA to the membrane
- Removal of residual contaminants
- Elution of pure genomic DNA

The columns have a high binding capacity and high quality DNA can be obtained from various species.

Principle:

HiPer® Blood Genomic DNA Extraction Teaching Kit (Column Based) simplifies the isolation of DNA from fresh blood with spin column procedure. 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 55°C which helps in the digestion of tissue and cell membranes. It is then applied onto HiElute Miniprep Spin Column which 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.
**Kit Contents:**

This kit can be used to extract genomic DNA from whole blood.

**Table 1: Enlists the materials provided in this kit with their quantity and recommended storage**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Product Code</th>
<th>Materials Provided</th>
<th>Quantity 10 expts</th>
<th>Quantity 20 expts</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TKC009</td>
<td>Control DNA</td>
<td>0.11 ml</td>
<td>0.22 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>2</td>
<td>DS0010</td>
<td>Lysis Solution (C1)</td>
<td>2.5 ml</td>
<td>5 ml</td>
<td>RT</td>
</tr>
<tr>
<td>3</td>
<td>DS0011</td>
<td>Prewash Solution</td>
<td>6 ml</td>
<td>12 ml</td>
<td>RT</td>
</tr>
<tr>
<td>4</td>
<td>DS0012</td>
<td>Wash Solution</td>
<td>6 ml</td>
<td>12 ml</td>
<td>RT</td>
</tr>
<tr>
<td>5</td>
<td>DS0040</td>
<td>Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]</td>
<td>2.5 ml</td>
<td>5 ml</td>
<td>RT</td>
</tr>
<tr>
<td>6</td>
<td>DS0013</td>
<td>Proteinase K Solution</td>
<td>0.25 ml</td>
<td>0.5 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>7</td>
<td>DS0003</td>
<td>RNase A Solution</td>
<td>0.25 ml</td>
<td>0.5 ml</td>
<td>RT</td>
</tr>
<tr>
<td>8</td>
<td>MB002</td>
<td>Agarose</td>
<td>4.8 g</td>
<td>9.6 g</td>
<td>RT</td>
</tr>
<tr>
<td>9</td>
<td>ML016</td>
<td>50X TAE</td>
<td>120 ml</td>
<td>240 ml</td>
<td>RT</td>
</tr>
<tr>
<td>10</td>
<td>ML015</td>
<td>6X Gel Loading Buffer</td>
<td>0.05 ml</td>
<td>0.1 ml</td>
<td>2-8°C</td>
</tr>
<tr>
<td>11</td>
<td>DBCA03</td>
<td>HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]</td>
<td>10 Nos.</td>
<td>20 Nos.</td>
<td>RT</td>
</tr>
<tr>
<td>13</td>
<td>DBCA016</td>
<td>Collection Tubes(Uncapped), Polypropylene (2.0 ml)</td>
<td>10 Nos.</td>
<td>20 Nos</td>
<td>RT</td>
</tr>
<tr>
<td>13</td>
<td>PW1139</td>
<td>Collection Tubes, Polypropylene (2.0 ml)</td>
<td>20 Nos.</td>
<td>40 Nos</td>
<td>RT</td>
</tr>
</tbody>
</table>

**Materials Required But Not Provided:**

**Glasswares:** Conical flask, Measuring cylinder, Beaker

**Reagents:** Distilled water, Ethidium bromide (10 mg/ml), Ethanol

**Other requirements:** Fresh whole blood, Electrophoresis apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Tabletop Micro centrifuge (with rotor for 2.0 ml tubes), Micropipettes, Tips, Adhesive tape, Microwave/Burner/Hotplate

**Storage:**

HiPer® Blood Genomic DNA Extraction Teaching Kit (Column Based) is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store the Control DNA and Proteinase K at -20°C. 6X Gel Loading Buffer should be stored at 2-8 °C. Other kit contents can be stored at room temperature (15-25°C).

**Important Instructions:**

1. Preheat a water bath or heating block to 55°C.
2. Thoroughly mix the 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 it to cool down to room temperature (15-25°C) before use.
3. Ensure the use of only clean & dry eppendorf tubes and tips for the procedure.
4. Ensure that the blood is collected under sterile conditions in an anticoagulant coated tube (e.g. EDTA).
5. Ensure that proper / appropriate precautions are taken while handling blood such as wearing nitrile gloves, covering mouth with face mask etc.
**Procedure:**

Read the Important Instructions before starting the experiment.

1. Take 200 μl of the fresh whole blood in a 2.0 ml collection tube. Ensure that the blood sample is at room temperature (15-25°C) before beginning the protocol.

2. Add 20 μl of Proteinase K solution into the above collection tube containing blood. Vortex (10-15 seconds) to ensure thorough mixing of the enzyme.

3. Add 20 μl of RNase A solution. Vortex (10-15 seconds) to ensure thorough mixing of the enzyme and incubate for 2 minutes at room temperature.

   **NOTE:** This step helps in getting RNA-free genomic DNA.

4. **Lysis Reaction**
   Add 200 μl of Lysis Solution to the sample, vortex thoroughly for few seconds to obtain a homogenous mixture. Incubate at 55°C for 10 minutes.

   **NOTE:** If cell clumps are visible, pipette the sample gently to obtain a homogenous mixture.

5. **Prepare for Binding**
   Add 200 μl of ethanol to the lysate obtained from step 4 and mix thoroughly by gentle pipetting for 5-10 seconds.

   **NOTE:** A homogenous solution should be obtained after addition of ethanol.

6. **Load lysate in HiElute Miniprep Spin Column**
   Transfer the entire lysate obtained from step 5 into HiElute Miniprep Spin Column. Centrifuge at 10,000 rpm for 1 minute. Discard the flow-through liquid and place the column in a new 2.0 ml collection tube.

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

7. **Prewash**
   Add 500 μl of Prewash Solution to the HiElute Miniprep Spin Column and centrifuge at 10,000 rpm for 1 minute. Discard the flow-through. Place the column in the same collection tube and centrifuge it for an additional one minute at 14,000 rpm to remove the traces of Wash Solution. Discard the collection tube and place the column in a new 2.0 ml collection tube.

8. **Wash**
   Add 500 μl of Wash Solution to the column and centrifuge at 14,000 rpm for 3 minutes. Discard the flow through. Place the column in the same collection tube and centrifuge it for an additional one minute at 14,000 rpm to remove the traces of Wash Solution. Discard the collection tube and place the column in a new 2.0 ml collection tube.

9. **DNA Elution**
   Add 200 μl of Elution Buffer directly into the column without spilling on 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.

   **Storage of the eluate with purified DNA:** The eluate contains pure genomic DNA. For short term storage (24-48 hours) 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.
**Agarose Gel Electrophoresis:**

**Preparation of 1X TAE:** To prepare 500 ml of 1X TAE buffer add 10 ml of 50X TAE buffer to 490 ml of sterile distilled water*. Mix well before use.

**Preparation of agarose gel:** To prepare 50 ml of 0.8% agarose gel, add 0.4 g agarose to 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate or burner by swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool down to about 55-60°C. Add 0.5 μl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

**NOTE:** Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

**Loading of the DNA samples:** To prepare sample for electrophoresis, add 2 μl of 6X gel loading buffer to 10 μl of DNA sample. Mix well by pipetting and load the sample onto the well. Load the Control DNA after extracting the DNA sample.

**Electrophoresis:** Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

* Molecular biology grade water is recommended (Product code: ML024).

**Quantitation 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 background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of DNA is calculated by the following formula:

Concentration of DNA sample (μg/ml) = 200 x A_{260} x dilution factor
Flowchart:

**Sample Preparation**
- Add 20µl of Proteinase K to 200µl of whole blood, vortex for 10-15 seconds.
- Add 20 µl of RNase- A solution, vortex for 10-15 seconds.
- Incubate for 2 minutes at room temperature.

**Load Lysate**
- Add 200 µl of lysis solution and vortex
- Incubate at 55°C for 10 minutes
- Add 200 µl of ethanol, mix by pipetting
- Load entire lysate onto the column

**Binding DNA to the column**
- Spin for 1 minute at 10,000 rpm and discard flow through

**Wash to remove contaminants**
- Add 500µl Prewash Solution
- Spin at 10,000 rpm for 1 minute
- Discard flow through and add 500 µl Wash Solution
- Spin at 14,000 rpm for 3 minutes
- Discard flow through and spin for additional 1

**DNA Elution**
- Add 200 µl of Elution Buffer
- Incubate at room temperature for 1 minute
- Spin at 10,000 rpm for 1 minute to elute the DNA

**Pure DNA**
Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV transilluminator and calculate the yield and purity using UV Spectrophotometer.

Lane 1: Control DNA
Lane 2: Blood Genomic DNA
Lane 3: Blood Genomic DNA with RNA contamination

Table 2: Absorbance of the extracted genomic DNA at 260 nm and 280 nm

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>( A_{260} )</th>
<th>( A_{280} )</th>
<th>( A_{260}/A_{280} )</th>
<th>Concentration (( \mu g/ml ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculate the concentration of isolated DNA using following formula:

Concentration of DNA sample (\( \mu g/ml \)) = 200 \times \( A_{260} \) \times \text{dilution factor}
**Interpretation:**

The lanes 1 and 2 demonstrate that highly purified blood genomic DNA has been obtained with no visible RNA contamination when electrophoresed on agarose gel. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic DNA as shown in lane 3 since RNA being of lower molecular weight than DNA runs faster than the genomic DNA. RNA contamination is observed when the RNase treatment has either been skipped or not been carried out properly.

An absorbance of 1.0 at 260 nm corresponds to approximately 50 μg/ml of DNA. If the $A_{260}/A_{280}$ ratio is 1.6-1.9, then the isolated DNA sample is considered to be pure. If higher $A_{260}/A_{280}$ ratio is observed it indicates the possibility of RNA contamination.

**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>Presence of cell clumps/colored residue on the spin column after washing</td>
<td>Inefficient cell lysis due to improper mixing of the Lysis Solution with the blood sample</td>
<td>The sample and the Lysis Solution should be mixed thoroughly by pulse-vortexing</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</td>
</tr>
<tr>
<td>2</td>
<td>Poor or low genomic DNA recovery</td>
<td>Lysate/ethanol mixture is not homogenous</td>
<td>In order to obtain a homogenous solution, mix thoroughly by gentle pipetting for 5-10 seconds before adding to the HiElute Miniprep Spin Column</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNA elution is improper</td>
<td>Ensure that 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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eluate contains traces of Wash Solution</td>
<td>Spin the column for an additional 2 minutes to dry the membrane completely</td>
</tr>
<tr>
<td>3</td>
<td>Shearing of genomic DNA</td>
<td>The blood sample used is old, degraded</td>
<td>If the blood sample is old the eluate may yield degraded DNA. For best results fresh whole blood should be used</td>
</tr>
<tr>
<td>4</td>
<td>DNA comes out of the well while loading</td>
<td>Eluate contains traces of Wash Solution</td>
<td>Spin the column for an additional 2 minutes to dry the membrane completely</td>
</tr>
</tbody>
</table>
**Technical Assistance:**
At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com

Storage temperature

15°C-25°C

Do not use if package is damaged

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