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.

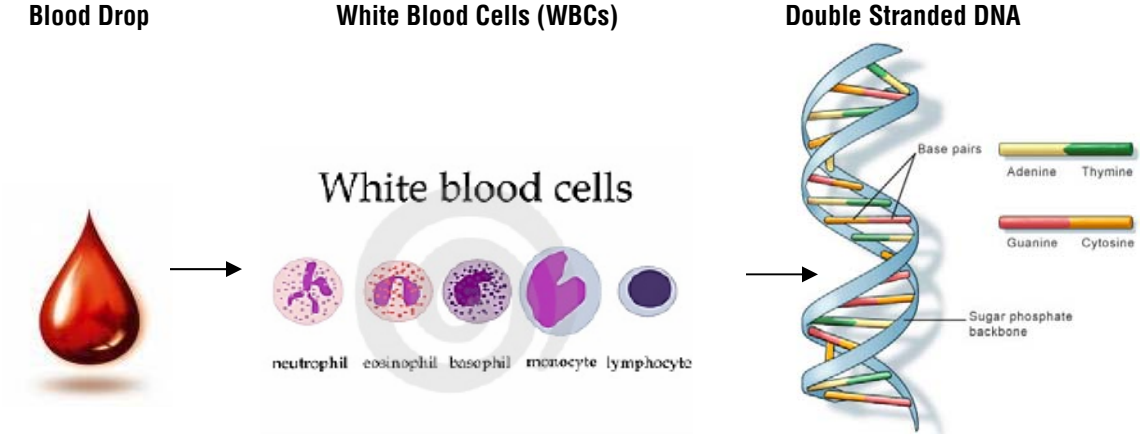


Fig 1: Extraction of blood genomic DNA from white blood cells (WBCs)

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

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

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 Ccolumn**
   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 liquid and re-use the same collection tube along with the column.

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 55-60ºC. 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.

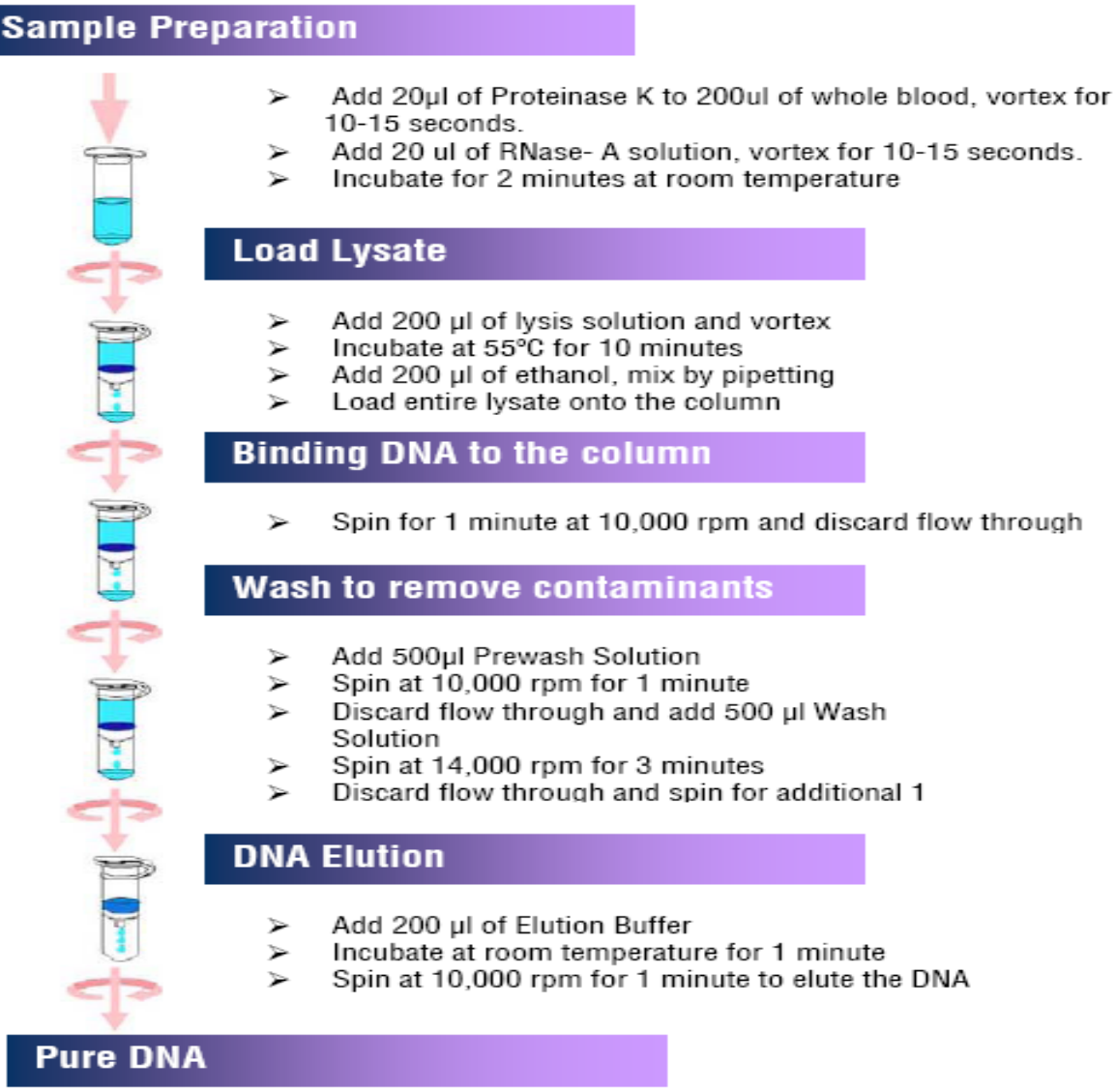
* Molecular biology grade water is recommended (Product code: ML024).

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 200ul of whole blood, vortex for 10-15 seconds.
➢ Add 20 ul 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.

Lanes **1** **2** **3**

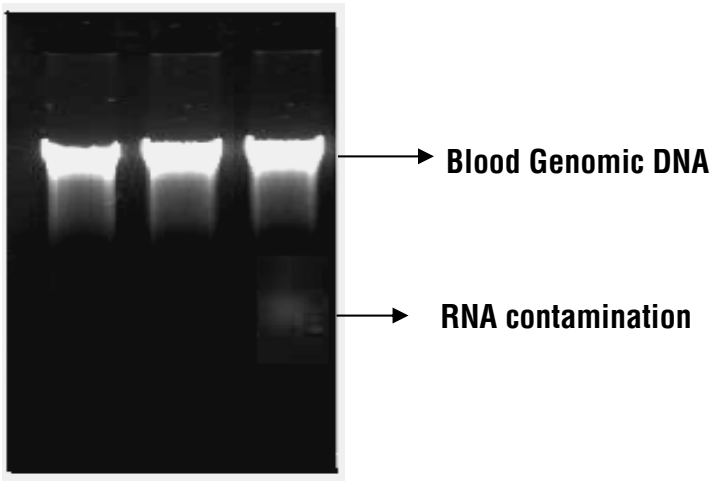


Fig 2: Gel image of isolated blood genomic DNA

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

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula:

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

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 Solution with the blood sample	The sample and the Lysis Solution should be mixed thoroughly by pulse-vortexing
		Due to decreased Proteinase K activity	Do not add Proteinase K directly to the Lysis Solution
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 for 5-10 seconds before adding to the HiElute Miniprep Spin Column
		DNA elution is improper	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
		Eluate contains traces of Wash Solution	Spin the column for an additional 2 minutes to dry the membrane completely
3	Shearing of genomic DNA	The blood sample used is old, degraded	If the blood sample is old the eluate may yield degraded DNA. For best results fresh whole blood should be used
4	DNA comes out of the well while loading	Eluate contains traces of Wash Solution	Spin the column for an additional 2 minutes to dry the membrane completely

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

Do not use if package is damaged

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