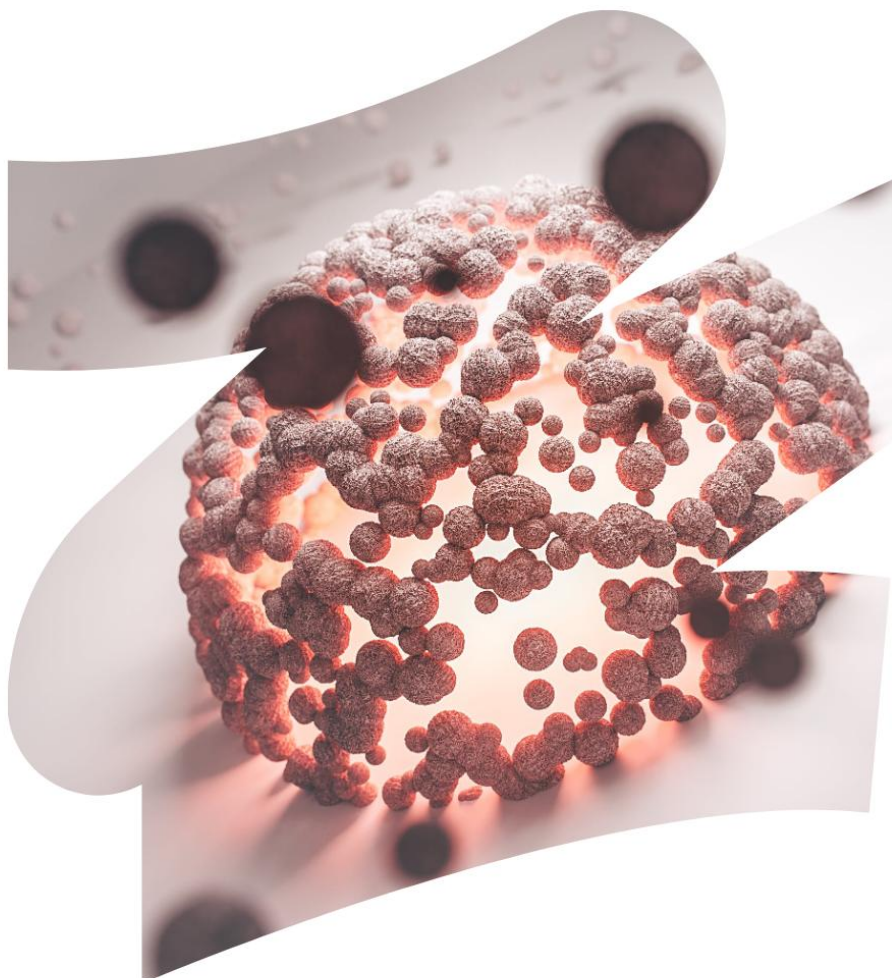


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A NEW GENERATION COMPANY



SN Blood Genomic DNA Extraction Teaching Kit

Solution Based

Innovation Speaks

SN Blood Genomic DNA Extraction Teaching Kit (Solution Based)

Product Code : SNLS - TK010

Number of experiments that can be performed :10

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 at -20°C
- Store RBC Lysis Buffer and 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 solution based method).

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 the 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.

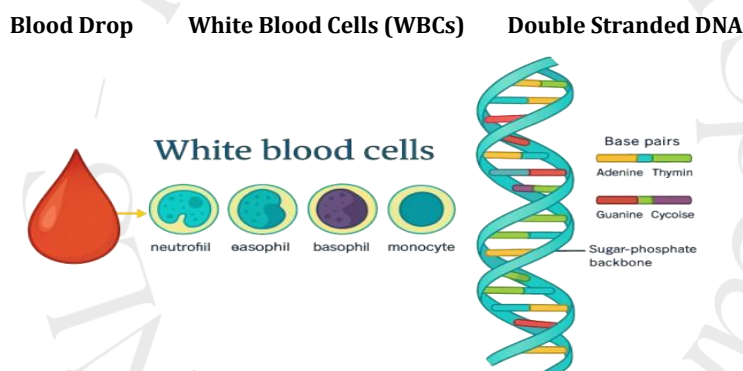


Fig 1: Extraction of blood genomic DNA from white blood cells (WBCs)

SN Blood Genomic DNA Extraction Teaching Kit (Solution Based) simplifies the isolation and purification of high molecular weight blood genomic DNA by using highly efficient solution based system. This kit does not contain harmful organic compounds such as phenol and chloroform.

The DNA Purification procedure comprises of the following steps:

- Lysis of RBC and WBC
- Protein Precipitation
- Precipitation of genomic DNA
- Removal of residual contaminants
- Elution of pure genomic DNA

Principle:

SN Blood Genomic DNA Extraction Teaching Kit (Solution Based) simplifies isolation and purification of high molecular weight genomic DNA from fresh whole blood by using highly efficient solution based system. Genomic DNA purification from whole blood involves lysis of the red blood cells with RBC Lysis Buffer followed by the lysis of white blood cells and their nuclei with WBC Lysis Buffer. Impurities like cellular

proteins are removed by precipitation and short washing steps while high molecular weight genomic DNA remains in the solution. High quality genomic DNA is then purified by isopropanol precipitation.

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.	Materials Provided	Quantity		Storage
		10 expts	20 expts	
1	Control DNA	0.11 ml	0.22 ml	-20°C
2	10X RBC Lysis Buffer	2 ml	4 ml	2-8°C
3	WBC Lysis Buffer (WBL)	4 ml	8 ml	RT
4	Precipitation Buffer (PBP)	1.2 ml	2.4 ml	RT
5	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	1.2 ml	2.4 ml	RT
6	RNase A Solution	0.02 ml	0.04 ml	RT
7	Isopropanol	4 ml	8 ml	RT
8	Agarose	4.8 g	9.6 g	RT
9	50XTAE	120 ml	240 ml	RT
10	6X Gel Loading Buffer	0.05 ml	0.1 ml	2-8°C
11	Collection Tubes, Polypropylene (2.0 ml)	30 Nos.	60 Nos.	RT

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker

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

Other requirements: 15 ml and 50 ml centrifuge tubes, Electrophoresis apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Tabletop Microcentrifuge (with rotor for 2.0 ml tubes), Micropipettes, Tips, Adhesive tape, Ice, Microwave/Burner/Hotplate

Storage:

SN Blood Genomic DNA Extraction Teaching Kit (Solution Based) is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store the Control DNA at -20°C and the 6X Gel Loading Buffer at 2-8°C. Other reagents can be stored at room temperature (15-25°C). WBC Lysis Buffer may form precipitate in cool ambient conditions. In such condition, heat the bottle before use at 55°C to dissolve the contents.

Important Instructions:

1. Preheat heating block or water bath to 65°C and 37°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 cooling 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. **Preparation of 1X RBC Lysis Buffer:** Add 18 ml of sterile distilled water to 2ml of 10XRBC Lysis Buffer
6. **Preparation of 70% Ethanol for 1 experiment:** Add 210 µl of ethanol (96-100%) to 90 µl of distilled water.

Procedure:

Read the Important Instructions before starting the experiment.

1. Take 300 μ l of 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. **RBC Lysis**
Add 900 μ l of RBC Lysis Buffer and mix well by inverting the tube for 6-8 times. Incubate at room temperature for 5 minutes. Mix the tube contents intermittently by inverting 2-3 times during incubation.
3. Centrifuge the tube at 15,000 rpm for 1 minute at room temperature. Discard the supernatant carefully without disturbing the white pellet such that very small amount (~15 μ l) of residue liquid remains back in the tube.

NOTE: If some red blood cells or cell debris are observed along with the white blood cell pellet, resuspend the white blood cell pellet in 600 μ l of 1X RBC Lysis Buffer. Incubate at room temperature for 2 minutes. Pellet down the white blood cells by repeating Step 3.

4. Vortex the tube vigorously so as to resuspend the white blood cells completely.
5. **WBC Lysis**
Add 300 μ l of WBC Lysis Buffer to the resuspended white blood cells and gently pipette to lyse the cells. Solution should become viscous. If any cell clumps are still present, incubate the solution at 37°C (10 minutes) until the clumps dissolve.
6. Add 1.5 μ l of RNase A solution. Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for 10 minutes at 37°C.
7. Cool the sample to room temperature before further processing.
8. **Precipitation of Proteins**
Add 100 μ l of Precipitation Buffer to the cell lysate. Mix by vortexing for 30 seconds. Incubate on ice for 5 minutes, as some protein clumps may be visible after vortexing.
9. Centrifuge at 14,000 rpm for 3 minutes at room temperature.

NOTE: Protein will precipitate and form a compact, dark, brown pellet. If the pellet is not compact then incubate on ice for 5 minutes and repeat the centrifugation as mentioned in Step 9.

10. **Precipitation of DNA**
Transfer the above supernatant to a new 2.0 ml collection tube. Add 300 μ l of 100% isopropanol and mix by inverting the tube gently till the DNA in white fibrous form is visible (30-40 times).
11. Centrifuge at 15,000 rpm for 1 minute at room temperature. Small white pellet of DNA will be visible. Discard the supernatant.
12. **Wash**
Remove the residual supernatant by carefully inverting the tube on a clean tissue paper without disturbing the pellet. Add 300 μ l of 70 % ethanol to the DNA pellet and wash by inverting the tube 6- 8 times.
13. Centrifuge at 15,000 rpm for 2 minutes at room temperature. Carefully discard the supernatant. The pellet may be very loose at this point, so the supernatant should be carefully discarded without disturbing the pellet. Repeat the wash step for one more time.

14. Invert the tube on a clean tissue paper and air-dry the pellet for 10-15 minutes.

15. DNA Elution

Add 100 µl of Elution Buffer and vortex for 1 minute. Incubate the tube at 65°C for 10 minutes to dissolve the DNA pellet completely.

Note: Sometimes fragments are seen after 10 minutes of incubation. In this case, increase the incubation time till the fragments dissolve completely. Some samples may need incubation for 1-2 hours at 65°C to rehydrate the DNA.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage of the DNA, 2-8°C and for long-term storage, -20°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 0.8% agarose gel, add 0.4 g agarose in 50 ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture in 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 0.5µl 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 into 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.

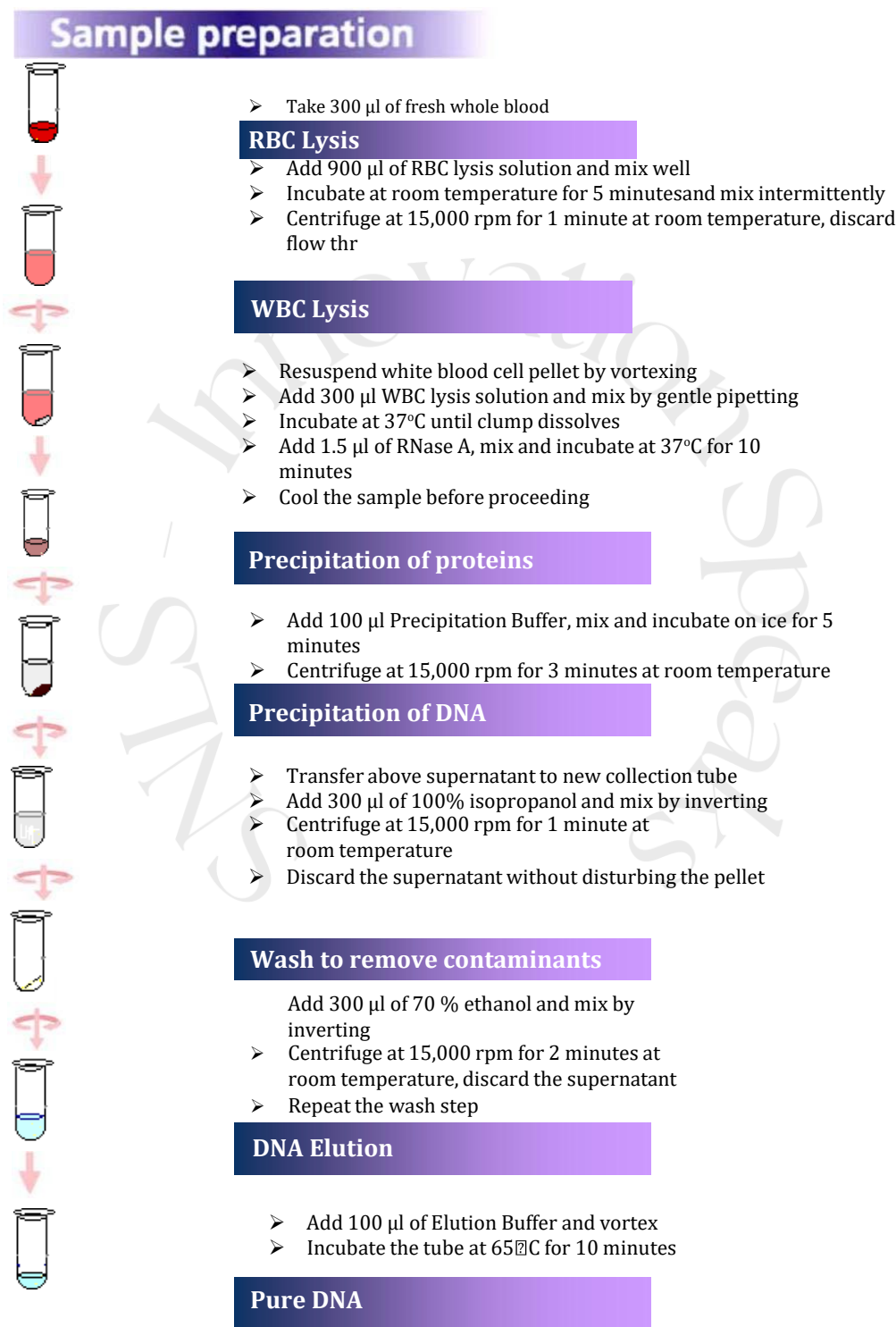
* Molecular biology grade water is recommended .

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) = 100 x A₂₆₀ x dilution factor

Flowchart:



Observation and Result:

Perform agarose gel electrophoresis after the completion of the DNA isolation procedure. Visualize the DNA bands using UV transilluminator and calculate the yield and purity using UV spectrophotometer.

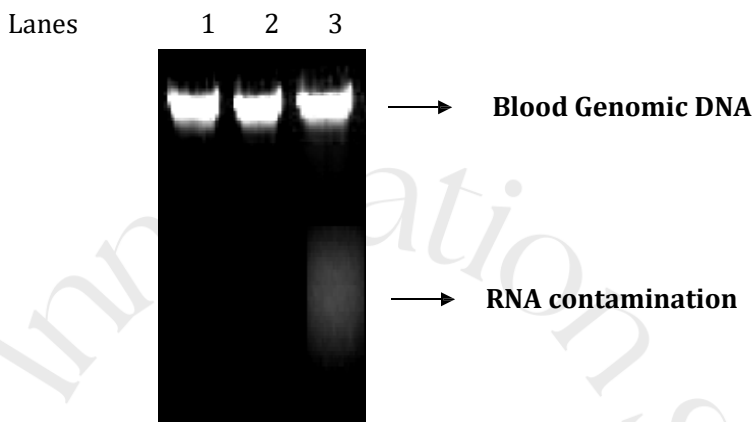


Fig 2: Gel image of genomic DNA extracted from blood sample

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) = 100 x A₂₆₀ 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 A₂₆₀/A₂₈₀ ratio is 1.6- 1.9, then the isolated DNA sample is considered to be pure. If higher A₂₆₀/A₂₈₀ ratio is observed it indicates the possibility of RNA contamination.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Low DNA recovery	Number of white blood cells is too less in the blood sample	Use fresh blood sample for every experiment
		White blood cells are not completely resuspended, before addition of WBC Lysis Buffer	Completely resuspend the white blood cells by vortexing vigorously
		DNA pellet was lost during isopropanol precipitation.	Be very careful while removing isopropanol or ethanol during precipitation and wash steps such that no DNA loss occurs
		The sample was not cooled to room temperature before adding Precipitation Buffer	Cool the sample to room temperature or chill the tube on ice for atleast 5 minutes and only then add Precipitation Buffer
2	Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is less)	Incomplete mixing with WBC Lysis Buffer leading to poor cell lysis	Repeat the procedure ensuring that the sample is vortexed immediately and completely with WBC Lysis Buffer
		Remains of hemoglobin present in the pellet	Repeat the procedure ensuring that enough volume of RBC Lysis Buffer is used. The white blood cell pellet should be white in colour
		Precipitation Buffer not mixed with WBC Lysis Buffer thoroughly	Ensure that Precipitation Buffer and cell lysate is mixed thoroughly
		DNA pellet was lost during isopropanol precipitation	Be very careful while removing isopropanol or ethanol during precipitation and wash steps such that no DNA loss occurs
3	No DNA eluted	DNA pellet has over dried	Rehydrate the DNA by incubating the DNA pellet at 65°C for 1 hour with Elution Buffer and then keep the sample overnight at 4°C
4	DNA pellet does not dissolve easily	During rehydration, DNA pellet was not mixed well	Shake well for few times during the rehydration step



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