

MB541

HiPurA® SPP Blood DNA Isolation Kit

Kit Contents

Product Code	Reagents provided	MB541			
		10 ML	50 ML	150 ML	300 ML
R075	RBC Lysis Buffer (10X RBL)	6 ml	30 ml	90 ml	180 ml
DS0046	WBC Lysis Buffer (WBL)	12 ml	60 ml	180 ml	360 ml
DS0047	Precipitation Buffer (PBP)	7 ml	35 ml	105 ml	210 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	2 ml	10 ml	30 ml	60 ml
DS0003	RNase A Solution (20 mg/ml)	100 µl	500 µl	1.5 ml	3 ml

Intended Use

Recommended for isolation of DNA from blood samples. For Research Use only.

Introduction

HiPurA® SPP Blood DNA Isolation Kit provide a fast and easy method for isolation of high molecular weight genomic DNA. These kits do not include any time consuming steps such as CsCl gradient ultracentrifugation and also eliminates the need for phenol-chloroform extraction. These kits also allow rapid processing of multiple samples within 90 minutes. The purified DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA® SPP Blood DNA Isolation Kit

The HiPurA® SPP Blood DNA Isolation Kit simplifies isolation of high molecular weight genomic DNA from fresh and anticoagulated whole blood by using highly efficient solution based system. This method can also be used for isolation of genomic DNA from bone marrow, buffy coat and cultured cells. Genomic DNA purification from whole blood involves lysis of red blood cells with RBC Lysis Buffer (R075) followed by lysis of white blood cells and their nuclei with WBL buffer (DS0046). 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. This system can be scaled up from 0.1ml to 12ml of whole blood.

Elution

The yield of genomic DNA depends on the sample type and the amount of starting sample. Elution is to be carried out by incubating the pellet obtained after processing, with sufficient amount of Elution Buffer (ET) (DS0040) at 65°C for 10 minutes. Some samples may need incubation at 65°C for 1- 2 hours. The volume of Elution Buffer to be used depends on volume of starting material. A single elution with Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. The eluted DNA is suitable for direct use in downstream applications such as 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 260nm, 280nm and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260nm corresponds to approximately 50µg/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 260nm to absorbance at 280nm. DNA purified by HiPurA® SPP Blood DNA Isolation 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.

DNA Yields from Various Starting Materials (depending on starting material)

Type of Starting Material	Amount of Starting Material	DNA Yield
Whole Blood (DNA Yield depends on quantity of white blood cells present)	50µl	0.3-0.6 µg
	100µl	1-5 µg
	200µl	3-10 µg
	300µl	5-15 µg
	500µl	7-23 µg
	600µl	10-30 µg
	800µl	12-35 µg
	1ml	15-48 µg
	2ml	30-90 µg
	3ml	50-150 µg
	4ml	65-200 µg
	5ml	100-300 µg
	10ml	150-600 µg
12ml	200-700 µg	
Buffy Coat	from 300µl blood	5-15 µg
	from 2ml blood	25-75 µg
Mouse Whole Blood	50µl	0.2-0.6 µg
	100µl	0.5-1.0 µg
	200µl	2-5 µg
	300µl	4-7 µg
Cultured Cells	2 X 10 ⁶ cells	10-15 µg

Materials needed but not provided

- 1X PBS (Product code: ML116) (for DNA Purification from Cultured Cells)
- 37°C water bath or heating block
- 65°C water bath or heating block
- Tabletop Microcentrifuge with rotor for 2.0ml tubes (For sample volume in a range of 100µl-1ml)
- Centrifuge with fixed angle rotor for 15ml tubes capable of $\geq 5,000 \times g$ (For sample volume in a range of 2ml - 6ml)
- Centrifuge with fixed angle rotor for 50 ml tubes capable of $\geq 5,000 \times g$ (For sample volume in a range of 7ml - 12ml)
- Nuclease-free 2.0 ml microcentrifuge tubes, 15ml and 50ml centrifuge tubes
- 70% Ethanol

- 100% Isopropanol
- Molecular Biology Grade Water (Product code: ML064)
- Clean absorbent paper towels
- Glycogen (20mg/ml) (For DNA Purification from clotted blood)
- Proteinase K Solution (25mg/ml) (Product code: MB086) (For DNA Purification from clotted Blood)

Storage

HiPurA® SPP Blood DNA Isolation Kit should be stored at 15-25°C for up to 18 months. Buffer WBL (DS0046) may form precipitate in cool ambient conditions. In such conditions, heat the bottle before use at 55°C, to dissolve the contents.

General Preparation Instructions

1. Preheat a heating block or a water bath to 65°C and 37°C.
2. **Thoroughly mix reagents:** Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
3. Ensure that clean & dry tubes and tips are used for the procedure.
4. **Dilute RBC Lysis Buffer (10X RBL) (R075) to working concentration (1X) as follows:**

Total volume of Blood	RBC Lysis Buffer (10X RBL)	Molecular Biology Grade Water (ML024)
10ml	6ml	54ml per bottle
50ml	30ml	270ml per bottle
150ml	90ml	810ml per bottle
300ml	180ml	1620ml per bottle

NOTE: It is recommended for the user to use sterile glass bottle for RBC Lysis buffer dilutions. Store the diluted RBC Lysis Buffer (1X) at 2-8°C.

Guidelines for Sample and Volume of Reagents to be used

The HiPurA® SPP Blood DNA Isolation Kit is a solution based system and the protocol can be easily modified depending on the sample volume. With the starting volume of 100µl whole blood, the user can go upto 12ml of whole blood. Accordingly, the volume of kit reagents to be used can be calculated based on the guidelines in the following table:

Sample Volume	Name of Solution	Solution Volume to be Used
1 X	1X RBL Buffer	3 X
1 X	WBL Buffer	1 X
1 X	PBP Buffer	0.33 X
1 X	Isopropanol	1 X
1 X	RNase A (Optional)	0.005 X
1 X	70% ethanol	1 X

Volume of Elution Buffer (ET) (DS0040) to be used can be adjusted depending on desired final concentration.

For sample volume in a range of 100µl - 1ml whole blood, nuclease-free 2.0ml microcentrifuge tubes are recommended. For larger volumes i.e. between 2ml-6ml whole blood, nuclease-free 15ml centrifuge tubes and for 12ml whole blood, nuclease-free 50ml centrifuge tubes are recommended.

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. 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 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

Procedure

A. DNA Purification Protocol for 300µl Whole Blood

1. Take initial volume 300µl of whole blood (or bone marrow) in clean a 2.0ml microcentrifuge tube.
2. **RBC Lysis**
Add 900µl of RBC Lysis Buffer (R075) (Refer General Preparation Instructions for dilution). Mix well by inverting the tube a few times. Incubate the tubes at room temperature (15-25°C) for 5 minutes. Mix the tube contents intermittently by inverting several times during incubation.
3. Centrifuge the tube at 10,000 x g (≈13,000 rpm) for 1 minute at room temperature (15-25°C). Discard the supernatant carefully without disturbing the white pellet such that only 15µl of residual liquid remains back in the tube. If the blood sample has been frozen, repeat steps 2-3 until pellet is white.

NOTE: If some red blood cells or cell debris are observed along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 600µl of RBC Lysis buffer (R075). Incubate

at room temperature (15-25°C) 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 300µl WBL Buffer (DS0046) to the resuspended white blood cells and pipet up and down to lyse the cells. Solution should become viscous.

NOTE: Incubate the solution at 37°C if any cell clumps are still present until all the clumps disappear.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 1.5µl of RNase A solution (20mg/ml) (DS0003). Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for 5-10 minutes at 37°C.

6. Cool the sample to room temperature (15-25°C) before further processing.

7. **Protein Precipitation**

Add 100µl PBP Buffer (DS0047) to the cell lysate. Mix by vortexing for 30 seconds at high speed. Incubate on ice for 5 minutes.

8. Centrifuge at maximum speed 10,000 x g (\approx 13,000 rpm) for 3 minutes at room temperature (15-25°C).

9. **DNA Precipitation**

Transfer the supernatant to a new 2.0ml microcentrifuge tube containing 300µl of 100% isopropanol. Mix by inverting the tube 30-40 times gently.

10. Centrifuge at 10,000 x g (\approx 13,000 rpm) for 1 minute at room temperature (15-25°C). Small white pellet of DNA will be visible.

11. **Wash**

Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 300µl of 70% ethanol, inverting the tube a few times.

12. Centrifuge at 10,000 x g (\approx 13,000 rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11-12 for second ethanol wash.

13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.

14. **DNA Elution**

Add 100µl of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 10 minutes. Some samples may need incubation for 1-2 hour at 65°C to rehydrate the DNA.

B. DNA Purification Protocol for 2ml Whole Blood

1. Take initial volume 2ml of whole blood (or bone marrow) in a clean 15ml centrifuge tube.

2. **RBC Lysis**

Add 6.0ml of RBC Lysis Buffer (R075) (Refer General Preparation Instructions for dilution). Mix well by inverting the tube a few times. Incubate the tubes at room temperature (15-25°C) for 5 minutes. Mix the tube contents intermittently by inverting several times during incubation.

3. Centrifuge the tube at 2,000 x g (\approx 5,000 rpm) for 5 minutes at room temperature (15-25°C). Discard the supernatant containing the lysed red blood cells carefully without disturbing the white pellet. Leave about 150 μ l of residual liquid in the tube. If the blood sample has been frozen, repeat steps 2-3 until pellet is white.

NOTE: If some red blood cells or cell debris are observed along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 4ml of RBC Lysis buffer (R075). Incubate at room temperature (15-25°C) 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 2ml WBL Buffer (DS0046) to the resuspended white blood cells and pipet up and down to lyse the cells. Solution should become viscous.

NOTE: Incubate the solution at 37°C if any cell clumps are still present until all the clumps disappear.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 10 μ l of RNase A Solution (20mg/ml) (DS0003). Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for approximately 10 minutes at 37°C.

6. Cool the sample to room temperature (15-25°C) before further processing.

7. **Protein Precipitation**

Add 670 μ l PBP Buffer (DS0047) to the cell lysate. Mix by vortexing for 30 seconds at high speed. Incubate on ice for 5 minutes.

8. Centrifuge at maximum speed \approx 2,000 x g (\approx 5,000 rpm) for 5 minutes at room temperature (15-25°C).

9. **DNA Precipitation**

Transfer the supernatant to a new 15ml centrifuge tube, containing 2ml of 100% isopropanol. Ensure that no protein pellet gets transferred along with the supernatant. Mix by inverting the tube 40-50 times gently.

10. Centrifuge at 2,000 x g (\approx 5,000 rpm) for 3 minutes at room temperature (15-25°C). A small white pellet of DNA will be visible.

11. **Wash**

Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 2ml of 70% ethanol, inverting the tube a few times.

12. Centrifuge at 2,000 x g (\approx 5,500 rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11-12 for second ethanol wash.

13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.

14. **DNA Elution**

Add 200 μ l of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight

to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.

C. DNA Purification Protocol for 12ml Whole Blood

1. Take initial volume 12ml of whole blood (or bone marrow) in a clean 50ml centrifuge tube.
2. **RBC Lysis**
Add 36ml of RBC Lysis Buffer (R075) (Refer General Preparation Instructions for dilution). Mix well by inverting the tube a few times. Incubate the tubes at room temperature (15-25°C) for 5 minutes. Mix the tube contents intermittently by inverting several times during incubation.
3. Centrifuge the tube at 2,000 x g (\approx 5,000 rpm) for 5 minutes at room temperature (15-25°C). Discard the supernatant containing the lysed red blood cells carefully without disturbing the white pellet. Leave about 350 μ l of residue liquid in the tube.

NOTE: If the blood sample has been frozen, repeat Steps 2-3 until pellet is white. If some red blood cells or cell debris are observed along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 24ml of RBC Lysis buffer (R075). Incubate at room temperature (15-25°C) 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 12ml WBL Buffer (DS0046) to the resuspended white blood cells and vortex for 2 minutes to lyse the cells. Solution should become viscous.

NOTE: Incubate the solution at 37°C if any cell clumps are still present until all the clumps disappear.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 50 μ l of RNase A solution (20mg/ml) (DS0003). Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for approximately 10 minutes at 37°C.

6. Cool the sample to room temperature (15-25°C) before further processing.
7. **Protein Precipitation**
Add 4ml PBP Buffer (DS0047) to the cell lysate. Mix by vortexing for 30 seconds at high speed. Incubate on ice for 5 minutes.
8. Centrifuge at maximum speed \approx 2,000 x g (\approx 5,000 rpm) for 10 minutes at room temperature (15-25°C).
9. **DNA Precipitation**
Transfer the supernatant to a new 50ml centrifuge tube, containing 12ml of 100% isopropanol. Ensure that no protein pellet gets transferred along with the supernatant. Mix by inverting the tube 40-50 times gently.
10. Centrifuge at 2,000 x g (\approx 5,000 rpm) for 5 minutes at room temperature (15-25°C). A small white pellet of DNA will be visible.
11. **Wash**
Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 12ml of 70% ethanol, inverting the tube a few times.

12. Centrifuge at 2,000 x g (\approx 5,000 rpm) for 5 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11-12 for second ethanol wash.

NOTE: If the resulting pellets are loose, centrifugation at higher speed or with prolonged time is recommended.

13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.

14. DNA Elution

Add 1ml of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.

D. DNA Purification Protocol for Buffy Coat (Prepared from 1- 1.5ml Whole Blood)

The buffy coat fraction of whole blood is enriched with WBC, and hence it gives at least 5-fold more DNA than from the same volume of blood. Buffy coat can be separated from whole blood by simply centrifuging the sample at 3,000- 4,000 x g (\approx 5,500-7,000 rpm) for 10 minutes at room temperature (15-25°C). Three layers will be obtained, such that plasma is in the upper layer, leucocytes in the middle layer (buffy coat), and erythrocytes in the bottom layer. The plasma can be aspirated off carefully ensuring that the layer of concentrated leukocytes is not disturbed. The buffy coat can then be collected carefully with a pipette and kept frozen at -70°C for long-term storage.

1. Add about 75-120 μ l buffy coat preparation (obtained from 1.5ml whole blood) into a nuclease-free 2ml microcentrifuge tube.

2. Lysis

Add 400 μ l of RBC Lysis Buffer (R075) (Refer General Preparation Instructions for dilution). Mix well by inverting the tube a few times. Incubate the tubes at room temperature (15-25°C) for 5 minutes. Mix the tube contents intermittently by inverting several times during incubation.

3. Centrifuge the tube at 10,000 x g (\approx 13,000 rpm) for 1 minute at room temperature (15-25°C). Discard the supernatant carefully without disturbing the white pellet. Leave about 15 μ l of residual liquid in the tube.

NOTE: If cell debris is observed along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 150-240 μ l volumes of RBC Lysis buffer (R075). Incubate at room temperature (15-25°C) 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 1.3ml WBL Buffer (DS0046) to the resuspended white blood cells and pipet up and down to lyse the cells. Solution should become viscous.

NOTE: Incubate the solution at 37°C if any cell clumps are still present until all the clumps disappear.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 5 μ l of RNase A solution (20mg/ml) (DS0003). Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for 5-10 minutes at 37°C.

6. Cool the sample to room temperature (15-25°C) before further processing.
 7. **Protein Precipitation**
Add 433µl PBP Buffer (DS0047) to the cell lysate. Mix by vortexing for 30 seconds at high speed. Incubate the tube in ice for 5 minutes.
 8. Centrifuge at maximum speed 10,000 x g (\approx 13,000 rpm) for 3 minutes at room temperature (15-25°C).
 9. **DNA Precipitation**
Transfer the supernatant to a new nuclease-free 2.0ml centrifuge tube containing 1.3ml of 100% isopropanol. Ensure that no protein pellet gets transferred along with the supernatant. Mix by inverting the tube 30-40 times gently.
 10. Centrifuge at 10,000 x g (\approx 13,000 rpm) for 1 minute at room temperature (15-25°C). A small white pellet of DNA will be visible.
 11. **Wash**
Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 1.3ml of 70% ethanol, inverting the tube a few times.
 12. Centrifuge at 10,000 x g (\approx 13,000 rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11-12 for second ethanol wash.
 13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.
 14. **DNA Elution**
Add 500µl of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.
- E. DNA Purification Protocol for Cultured Cells**
- This protocol is designed for isolating genomic DNA from 0.5- 1 x 10⁶ cultured cells.
1. **Harvest cells**
 - **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto 0.5- 1 x 10⁶ cultured cells for 5 minutes at 300 x g (\approx 1500 rpm). Discard the culture medium and continue with step 2.
 - **Suspension cell cultures:** Centrifuge upto 0.5- 1 x 10⁶ cultured cells for 5 minutes at 300 x g [\approx 1500 rpm]. Discard the culture medium completely and continue with step 2.

NOTE: Cells can be harvested, by aliquoting in 2.0ml microcentrifuge tubes and flash-freezing in liquid nitrogen, these can be stored at -70°C for several months before preparing DNA.
 2. Harvest the cells and resuspend them in 200µl 1X PBS (ML116) into a 2ml microcentrifuge tube.
 3. Centrifuge the tube at 10,000 x g (\approx 13,000 rpm) for 1 minute to pellet down the cells. Discard the supernatant and leave about 15µl of residual liquid in the tube.
 4. Vortex the tube vigorously so as to resuspend the cells in the residual liquid completely.
 5. **WBC Lysis**

Add 150µl WBL Buffer (DS0046) to the resuspended white blood cells and pipet up and down to lyse the cells. Solution should become viscous.

NOTE: Incubate the solution at 37°C if any cell clumps are still present until all the clumps disappear.

Optional RNase A treatment

If RNA-free genomic DNA is required, add 1µl of RNase A solution (20mg/ml) (DS0003). Invert the tube 20-25 times to ensure thorough mixing of enzyme and incubate for 5-10 minutes at 37°C.

6. Cool the sample to room temperature (15-25°C) before further processing.

7. Protein Precipitation

Add 50µl PBP Buffer (DS0047) to the cell lysate. Mix by vortexing for 30 seconds at high speed. Incubate the tube in ice for 5 minutes.

8. Centrifuge at maximum speed $\approx 10,000 \times g$ ($\approx 13,000$ rpm) for 3 minutes at room temperature (15-25°C).

9. DNA Precipitation

Transfer the supernatant to a new nuclease-free 2ml centrifuge tube containing 150µl of 100% isopropanol. Ensure that no protein pellet gets transferred along with the supernatant. Mix by inverting the tube 30-40 times gently.

10. Centrifuge at 10,000 x g ($\approx 13,000$ rpm) for 1 minute at room temperature (15-25°C). A small white pellet of DNA will be visible.

11. Wash

Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 150µl of 70% ethanol, inverting the tube a few times.

12. Centrifuge at 10,000 x g ($\approx 13,000$ rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 10-11 for second ethanol wash.

13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.

14. DNA Elution

Add 50µl of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.

F. DNA Purification Protocol for 50µl Clotted Blood

1. Transfer 50µl of clotted blood including any residual liquid into a 1.5ml microcentrifuge tube.

2. Lysis

Add 550µl WBL Buffer (DS0046) to the sample and pipet up and down few times to mix.

3. Proteinase K treatment

Add 3µl of Proteinase K Solution (25mg/ml) and mix thoroughly by inverting about 20 times.

4. Incubate at 55°C for 1 hour or overnight until clot dissolve completely.

5. Incubate the tube in ice for 1 minute.
 6. **RNase A treatment**
Add 3µl of RNase A solution (20mg/ml) (DS0003) to the cell lysate. Invert the tube 10 times to ensure thorough mixing of enzyme. Incubate for 5 minutes at 37°C.
 7. Incubate the tube in ice for 1 minute.
 8. **Protein Precipitation**
Add 200µl of PBP Buffer (DS0047) to the cell lysate after incubation in Step 7. Mix by vortexing for 30 seconds at high speed. Incubate the tube in ice for 5 minutes.
 9. Centrifuge at maximum speed ≈10,000 x g (≈13,000 rpm) for 3 minutes at room temperature (15-25°C).
 10. **DNA Precipitation**
Transfer the supernatant to a new nuclease-free 2ml centrifuge tube containing 600µl of 100% isopropanol. If the DNA yield is expected to be lower than 2µg, add 2µl of glycogen (20mg/ml) per tube. Mix by inverting the tube 30-40 times gently.
 11. Centrifuge at 10,000 x g (≈13,000 rpm) for 1 minute at room temperature (15-25°C). A small white pellet of DNA will be visible.
 12. **Wash**
Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 600µl of 70% ethanol, inverting the tube a few times.
 13. Centrifuge at 10,000 x g (≈13,000 rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 12-13 for second ethanol wash.
 14. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.
 15. **DNA Elution**
Add 20µl of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.
- G. DNA purification from 1ml Clotted Blood**
1. Transfer 1ml of clotted blood including any liquid residual into a 50ml tube.
 2. **WBC Lysis**
Add 11ml WBL Buffer (DS0046) to the sample and pipette up and down few times to mix.
 3. **Proteinase K treatment**
Add 62.5 µl of Proteinase K (20mg/ml) (DS0003) and mix thoroughly by inverting about 20 times. Incubate at 55°C for 3 hours or overnight until clots dissolve completely.
 4. Incubate the tube in ice for 1- 2 minutes.
 5. **RNase A treatment**
Add 50µl of RNase A solution (DS0003) to the cell lysate. Invert the tube 10 times to ensure thorough mixing of enzyme. Incubate for 5 minutes at 37°C.
 6. Incubate the tube in ice for 1- 2 minutes.

7. **Protein Precipitation**

Add 4ml of PBP Buffer (DS0047) to the cell lysate after incubation in Step 6. Mix by vortexing for 30 seconds at high speed. Incubate the tube in ice for 10 minutes.

8. Centrifuge at 2,000 x g (\approx 5,500 rpm) for 10 minutes at room temperature (15-25°C).

9. **DNA Precipitation**

Transfer the supernatant to a new nuclease-free 50ml centrifuge tube containing 12ml of 100% isopropanol. Add 20 μ l of glycogen (20mg/ml) per tube. Mix by inverting the tube 30- 40 times gently.

10. Centrifuge at 2,000 x g (\approx 5,500 rpm) for 5 minutes at room temperature (15-25°C). A small white pellet of DNA will be visible.

11. **Wash**

Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Wash the DNA pellet by adding 12ml of 70% ethanol, inverting the tube a few times.

12. Centrifuge at 2,000 x g (\approx 5,500 rpm) for 2 minutes at room temperature (15-25°C). Carefully pour off the ethanol. The pellet may be very loose at this point, so the supernatant should be carefully poured off without disturbing the pellet. Repeat steps 11-12 for second ethanol wash.

13. Invert the tube on a paper towel and air-dry the pellet for 10-15 minutes.

14. **DNA Elution**

Add 400 μ l of Elution Buffer (ET) (DS0040) and vortex for 1 minute to dissolve the DNA pellet properly. Incubate the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight to rehydrate the DNA. Gently shake the tube several times intermittently during the incubation to dissolve the DNA completely.

H. DNA purification from large volume (>1ml) Clotted Blood

1. Transfer the clotted blood including any residual liquid into a 50ml tube. Homogenize the sample with a rotor- stator homogenizer until the sample is uniformly homogenous.

2. **RBC Lysis**

Add 3 volumes of 1X RBC Lysis Buffer (R075) and mix by inverting the tube 5-7 times. Incubate the cell lysate at room temperature (15-25°C) for 5 minutes.

3. Centrifuge at 2,000 x g (\approx 5,000 rpm) for 5 minutes. Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel. Make sure that the pellet remains in the tube.

4. **WBC Lysis**

Add 5ml WBL Buffer (DS0046) to the sample and add 50 μ l of Proteinase K Solution (25mg/ml). Close the cap and vortex immediately such that the pellet is completely homogenized.

NOTE: When processing multiple samples, each tube should be vortexed immediately after addition of WBL Buffer (DS0046)/Proteinase K Solution. Do not wait till buffer is added to all the tubes before vortexing. The pellet can be easily homogenized with 3-4 pulses of high-speed vortexing, but some traces of pellet with a jelly-like consistency may remain. If these traces are seen, the samples should be vortexed for another 30 seconds.

5. Incubate at 65°C for 30 minutes in a water bath or heating block and cool the lysate to room temperature (15-25°C).

6. Protein Precipitation

Add 1.7ml of PBP Buffer (DS0047) to the lysate and mix by vortexing for 15 seconds. Incubate the tube in ice for 10 minutes.

7. Centrifuge at 2,000-4,000 x g (\approx 5,000- 8,000 rpm) for 10 minutes to pellet down the protein.

8. DNA Precipitation

Transfer the supernatant from step 7 to a new 50ml tube. Add 4.75ml of isopropanol and mix gently by inverting the tube 20-30 times. DNA precipitate will become visible as threads or clumps.

9. Centrifuge at 2,000-4,000 x g (\approx 5,000-8,000 rpm) for 5-10 minutes to pellet down the DNA. Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel.

10. Wash

Wash the DNA pellet by adding 5ml of 70% ethanol and vortexing for 10 seconds.

11. Centrifuge at 2,000-4,000 x g (\approx 5,000-8,000 rpm) for 5-10 minutes to pellet down the DNA. Repeat step 10-11 for second ethanol wash.
12. Discard the supernatant and dry the pellet by inverting the tube on a clean absorbent paper towel for 5 minutes.

NOTE: Air-dry the pellet till all the liquid evaporates, but ensure that the pellet does not over-dry because it becomes very difficult to dissolve the over-dried pellet.

13. DNA Elution

Add 1ml of Elution Buffer (ET) (DS0040) and vortex for 5 seconds at low speed. Dissolve the DNA pellet completely by incubating the tube at 65°C for 1 hour and at room temperature (15-25°C) overnight 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.

Warning and Precautions

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

Type of Sample	DNA Yield	DNA Purity
Whole blood	4-12 μ g	1.6-1.9

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Low DNA recovery	Number of white blood cells is too less in the blood sample	Draw new blood sample every time.
		Blood sample is too old	Try to use fresh blood sample every time, if possible.
		White blood cells are not completely resuspended, before addition of WBL 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.
2.	Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is less)	The sample was not cooled to room temperature (15-25°C) before adding Buffer PBP	Cool the sample to room temperature (15-25°C) or chill the tube on ice for atleast 5 minutes and only then add Buffer PBP.
		Incomplete mixing with Buffer WBL leading to poor cell lysis	Repeat the procedure ensuring that the sample is vortexed immediately and completely with Buffer WBL.
		Remains of hemoglobin present in the WBC pellet	Repeat the procedure ensuring that enough volume of RBC Lysis Buffer is used. The white blood cell pellet should be white in colour.
		Buffer PBP not mixed with cell lysate thoroughly	Ensure that Buffer PBP and cell lysate is mixed thoroughly.
3.	No DNA eluted.	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.
4.	DNA pellet does not dissolve easily	DNA pellet has over dried	Rehydrate the DNA by incubating the DNA pellet at 65°C for 1 hour with Elution Buffer (ET) and then keep the sample at 4°C overnight.
		During rehydration, DNA pellet was not mixed well	Shake well for few times during the rehydration step.

Safety Information

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.

Please refer disclaimer Overleaf.

Symbols

	Manufacturer		Do not use if package is damaged
	Contains biological material of animal origin		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Batch code
	Catalogue number		

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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.

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