

PROTOCOLS FOR GENOMIC DNA ISOLATION WITH THE PROMEGA WIZARD GENOMIC DNA PURIFICATION KIT (CAT #A1120)

Promega tested the purification of genomic DNA from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes and detected no adverse effects upon subsequent manipulations of the DNA, including PCR. Anticoagulant blood samples may be stored at 2-8°C for up to two months, but DNA yield will be reduced with increasing length of storage.

The protocol in Section III.A has been designed and tested for blood samples up to 3ml in volume. The protocol in Section III.B has been designed and tested for blood samples up to 10ml in volume. The yield of genomic DNA will vary depending on the quantity of white blood cells present. Frozen blood may be used in the following protocols, but yield may be lower than that obtained using fresh blood, and additional Cell Lysis Solution may be required.

III.A. Isolating Genomic DNA from Whole Blood

(300µl or 3ml Sample Volume)

Materials to Be Supplied by the User

- . sterile 1.5ml microcentrifuge tubes (for 300µl blood samples)
- . sterile 15ml centrifuge tubes (for 3ml blood samples)
- . water bath, 37°C
- . isopropanol, room temperature
- . 70% ethanol, room temperature
- . water bath, 65°C (optional, for rapid DNA rehydration)

1. **For 300µl Sample Volume:** Add 900µl of Cell Lysis Solution to a sterile 1.5ml microcentrifuge tube. **For 3ml Sample Volume:** Add 9.0ml of Cell Lysis Solution to a sterile 15ml centrifuge tube.

Important: Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer blood to the tube containing the Cell Lysis Solution. Invert the tube 5 to 6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2 to 3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000 to 16,000 × g for 20 seconds at room temperature for 300µl sample. Centrifuge at 2,000 × g for 10 minutes at room temperature for 3ml sample.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 10 to 20µl of residual liquid will remain in the 1.5ml tube

(300µl sample). Approximately 50 to 100µl of residual liquid will remain in the 15ml tube (3ml sample).

If blood sample has been frozen, repeat Steps 1 to 4 until pellet is white. There may be some loss of DNA from frozen samples.

Note: Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears to contain only red blood cells, add an additional aliquot of Cell Lysis Solution after removing the supernatant above the cell pellet, and then repeat **Steps 3 & 4**.

5. Vortex the tube vigorously until the white blood cells are resuspended (10 to 15 seconds).

Completely resuspend the white blood cells to obtain efficient cell lysis.

6. Add Nuclei Lysis Solution (300µl for 300µl sample volume; 3.0ml for 3ml sample volume) to the tube containing the resuspended cells. Pipet the solution 5 to 6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disrupted. If the clumps are still visible after 1 hour, add additional Nuclei Lysis Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) and repeat the incubation.

7. **Optional:** Add RNase Solution (1.5µl for 300µl sample volume; 15µl for 3ml sample volume) to the nuclear lysate and mix the sample by inverting the tube 2 to 5 times. Incubate the mixture at 37°C for 15 minutes, and then cool to room temperature.

8. Add Protein Precipitation Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) to the nuclear lysate and vortex vigorously for 10 to 20 seconds. Small protein clumps may be visible after vortexing.

Note: If additional Nuclei Lysis Solution was added in **Step 6**, add a total of 130µl Protein Precipitation Solution for 300µl sample volume and 1.3ml Protein Precipitation Solution for 3ml sample volume.

9. Centrifuge at 13,000 to 16,000 × g for 3 minutes at room temperature for 300µl sample volume. Centrifuge at 2,000 × g for 10 minutes at room temperature for 3ml sample volume. A dark brown protein pellet should be visible.

10. For 300µl sample volume, transfer the supernatant to a clean 1.5ml microcentrifuge tube containing 300µl of room temperature isopropanol. For 3ml sample volume, transfer the supernatant to a 15ml centrifuge tube containing 3ml room temperature isopropanol.

Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuge at $13,000$ to $16,000 \times g$ for 1 minute at room temperature for $300\mu\text{l}$ sample. Centrifuge at $2,000 \times g$ for 1 minute at room temperature for 3ml sample. The DNA will be visible as a small white pellet.

13. Decant the supernatant, and add one sample volume of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as in Step 12.

14. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-dry the pellet for 10 to 15 minutes.

15. Add DNA Rehydration Solution ($100\mu\text{l}$ for $300\mu\text{l}$ sample volume; $250\mu\text{l}$ for 3ml sample volume) to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C .

16. Store the DNA at $2-8^{\circ}\text{C}$.

III.B. Isolating Genomic DNA from Whole Blood (10ml Sample Volume)

Materials to Be Supplied by the User

- . sterile 50ml centrifuge tubes
- . water bath, 37°C
- . isopropanol, room temperature
- . 70% ethanol, room temperature
- . water bath, 65°C (optional; for rapid DNA rehydration)

1. **For 10ml whole blood samples:** Add 30ml of Cell Lysis Solution to a sterile 50ml centrifuge tube.

Important: Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer 10ml of blood to the tube containing the Cell Lysis Solution. Invert the tube 5 to 6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2 to 3 times once during the incubation) to lyse the red blood cells. Centrifuge at $2,000 \times g$ for 10 minutes at room temperature.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 1 to 4ml of residual liquid will remain. If blood sample has been frozen, add an additional 30ml of Cell Lysis Solution, invert 5 to 6 times to mix, and repeat Steps 3 & 4 until pellet is nearly white. There may be some loss of DNA in frozen samples.

Note: Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears to contain only red blood cells, add an additional aliquot of Cell Lysis Solution after removing the supernatant above the cell pellet, and then repeat **Steps 3 & 4**.

5. Vortex the tube vigorously until the white blood cells are resuspended (10 to 15 seconds).

Completely resuspend the white blood cells to obtain efficient cell lysis.

6. Add 10ml of Nuclei Lysis Solution to the tube containing the resuspended cells. Pipet the solution 5 to 6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disrupted. If the clumps are still visible after 1 hour, add 3ml of additional Nuclei Lysis Solution and repeat the incubation.

7. **Optional:** Add RNase A, to a final concentration of 20µg/ml, to the nuclear lysate and mix the sample by inverting the tube 2 to 5 times. Incubate the mixture at 37°C for 15 minutes, and then cool to room temperature.

8. Add 3.3ml of Protein Precipitation Solution to the nuclear lysate and vortex vigorously for 10 to 20 seconds. Small protein clumps may be visible after vortexing.

Note: If additional Nuclei Lysis Solution was added in **Step 6**, add 4ml of Protein Precipitation Solution (instead of 3.3ml).

9. Centrifuge at 2,000 × *g* for 10 minutes at room temperature. A dark brown protein pellet should be visible.

10. Transfer the supernatant to a 50ml centrifuge tube containing 10ml of room temperature isopropanol.

Note: Some supernatant may remain in the original tube containing the protein pellet. Leave the residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuge at $2,000 \times g$ for 1 minute at room temperature. The DNA will be visible as a small white pellet.

13. Decant the supernatant and add 10ml of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the centrifuge tube. Centrifuge as in **Step 12**.

14. Carefully aspirate the ethanol. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Air dry the pellet for 10 to 15 minutes.

15. Add 800 μ l of DNA Rehydration Solution to the tube, and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

16. Store the DNA at 2-8°C.

III.C. Isolating Genomic DNA from Whole Blood (96-well plate)

This protocol can be scaled to 20 μ l, 30 μ l or 40 μ l of blood. Table 2 outlines the various solution volumes used in each step. Fifty-microliter preps generally yield genomic DNA in the range of 0.2 to 0.7 μ g, depending upon the number of leukocytes in the blood sample.

Table 2. Volumes of Reagents Required for Various Starting Amounts of Blood.

Sample	Cell Lysis Solution (RBC lysis)	Nucleic Lysis Solution	Protein Precipitation Solution	Isopropanol	DNA Rehydration Solution
20 μ L	60 μ L	20 μ L	6.7 μ L	20 μ L	10 μ L
30 μ L	90 μ L	30 μ L	10 μ L	30 μ L	15 μ L
40 μ L	120 μ L	40 μ L	13.3 μ L	40 μ L	20 μ L
50 μ L	150 μ L	50 μ L	16.5 μ L	50 μ L	25 μ L

Materials to Be Supplied by the User

- . V-bottom 96-well plate(s) able to hold 300 μ l volume/well (Costar® Cat.# 3896)
- . isopropanol, room temperature
- . 70% ethanol, room temperature
- . 96-well plate sealers (Costar® Cat.# 3095) (optional; for use with human blood)

1. Add 150 μ l Cell Lysis Solution to each well.

Important: Blood must be collected in EDTA, heparin or citrate anticoagulant tubes.

2. Add 50µl of fresh blood to each well and pipet 2 to 3 times to mix.
3. Leave the plate at room temperature for 10 minutes, pipetting the solution twice during the incubation to help lyse the red blood cells.
4. Centrifuge at $800 \times g$ for 5 minutes in a tabletop centrifuge to concentrate the cells.
5. Carefully remove and discard as much of the supernatant as possible with a micropipette tip, leaving a small pellet of white cells and some red blood cells. The use of an extended pipette tip, such as a gel loading tip, is recommended. Tilting the 96-well plate 50 to 80° (depending on the amount of liquid present per well) allows more thorough removal of liquid from the well.
6. Add 50µl of Nuclei Lysis Solution to each well and pipet 5 to 6 times to resuspend the pellet and lyse the white blood cells. The solution should become more viscous. As an aid in DNA pellet visualization, 2µl per well of a carrier (e.g., Polyacryl Carrier [Molecular Research Center, Inc., Cat.# PC152]) can be added at this step. DNA yields are generally equivalent with or without carrier use.
7. Add 16.5µl of Protein Precipitation Solution per well and pipet 5 to 6 times to mix.
8. Centrifuge at $1,400 \times g$ for 10 minutes at room temperature. A brown protein pellet should be visible.
9. DNA Precipitation/Rehydration in 96-Well Plate:
 - a. Carefully transfer the supernatants to clean wells containing 50µl per well of room temperature isopropanol and mix by pipetting.

Note: Some of supernatant may remain in the original well containing the protein pellet. Leave this residual liquid in the well to avoid contaminating the DNA solution with the precipitated protein. As in **Step 5**, tilting the plate will facilitate removal of liquid from the well. Using an extended pipette tip in this step does not allow easy sample mixing with isopropanol.
 - b. Centrifuge at $1,400 \times g$ for 10 minutes. Carefully remove the isopropanol with a micropipette tip.
 - c. Add 100µl of room temperature 70% ethanol per well.
 - d. Centrifuge at $1,400 \times g$ for 10 minutes at room temperature.
 - e. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. Care must be taken to avoid aspirating the DNA pellet. Place the tray at a 30 to 45° angle and air-dry for 10 to 15 minutes.

f. Add 25µl of DNA Rehydration Solution to each well. Allow the DNA to rehydrate overnight at room temperature or at 4°C.

g. Store the DNA at 2-8°C.

Note: Small volumes of DNA can be easily collected at the bottom of a V-well by briefly centrifuging the 96-well plate before use.