

AxyPrep Blood Genomic DNA Midiprep Kit

For the purification of genomic DNA from whole blood

Kit contents, storage and stability

Cat. No.	AP-MD-BL-GDNA-10	AP-MD-BL-GDNA-25
Kit size	10 preps	25 preps
Midiprep column	10	25
Column adapter	10	25
Midiprep syringe filter	10	25
Plastic wrench	1	1
Midi-column cap	10	25
Buffer VL	72 ml	180 ml
Buffer G-B	72 ml	180 ml
Buffer DV bottle (empty)	1	1
Buffer DV-A	5 ml	10 ml
Buffer BV	72 ml	180 ml
Buffer W1	96 ml	2 × 120 ml
Buffer W2 concentrate	36 ml	2 × 72 ml
Eluent	10 ml	20 ml
Protocol manual	1	1

All kit components are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Buffer VL: Cell and virus lysis buffer. Store at room temperature.

Buffer G-B: Protein-denaturing buffer. Store at room temperature.

Buffer DV-A: Buffer DV additive. Used for preparation of Buffer DV (refer to Preparation before experiment on page 4 for details). Store at room temperature.

Buffer DV: Phase partition buffer. Store at room temperature

Buffer BV: Nucleic acid binding buffer. Store at room temperature

Buffer W1: Wash buffer. Store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before use, add ethanol as much as indicated on the bottle, and mix well. Store at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5. Store at room temperature.

Introduction

The AxyPrep Blood Genomic DNA Midiprep Kit is designed for the isolation of up to 100 µg of high molecular weight genomic DNA from up to 3 ml of anticoagulated human or animal whole blood or up to 120 µl of anticoagulated avian or amphibian whole blood. The genomic DNA produced by this procedure will also include mitochondrial DNA and viral DNA. The purification of genomic DNA with this kit is based on a unique two-phase partitioning technique, in combination with the selective binding of DNA to a special AxyPrep column. The optimized buffers used in this kit ensure that only nucleic acids will be adsorbed onto the AxyPrep column, while cellular proteins, metabolites and PCR inhibitors are removed. The purified genomic DNA is approximately ≥ 30 kb in size, free from contaminating proteins, polysaccharides, lipids and pigments, and is ideally suited for use in PCR applications.

Comments about the various protocol steps

1. Amount of starting material (blood volume)

Under different physiologic states, the number of leukocytes in human and animal whole blood may vary greatly (Table 1). The number of leukocytes per ml of "normal" human or animal whole blood (ie., "leukocyte count") is defined as a baseline value for this kit. In the case of immunosuppression or aplastic anemia, the number of leukocytes will typically be lower. The maximum volume of blood processed should not be increased in an attempt to compensate for a diminished leukocyte count due to limitations of the purification system to assimilate RBCs. Conversely, during infection and leukemia, the leukocyte count is higher than normal and the volume of the blood sample should be scaled down to avoid overwhelming the procedure with excessive WBC debris and DNA.

Table1: Leukocytes/ml of human whole blood under different physiologic states

Physiologic condition	Leukocyte count
Normal	$4-7 \times 10^6$
Immunosuppression or aplastic anemia	2×10^6
Inflammation	4×10^7
Leukemia	5×10^8

2. Release of genomic DNA

Buffer VL lyses blood cells, virus and mitochondria directly without the need to separate out RBCs in advance. Buffer VL will also kill any blood-borne viruses present. The RBCs and WBCs are lysed by vigorous vortexing or by passage 6-8× through a 18-gauge syringe needle. This mechanical shearing in the presence of Buffer VL will achieve full release of the WBC genomic DNA, mitochondrial DNA, viral DNA and viral RNA. To avoid contamination of work surfaces and possible infection of laboratory personnel, be particularly careful not to splash the lysate outside the tube, always wear a labcoat, gloves and protective glasses when handling blood samples. Following lysis, the addition of Buffer G-B, denatures and aggregates all cellular protein, leaving the genomic DNA soluble and free in solution.

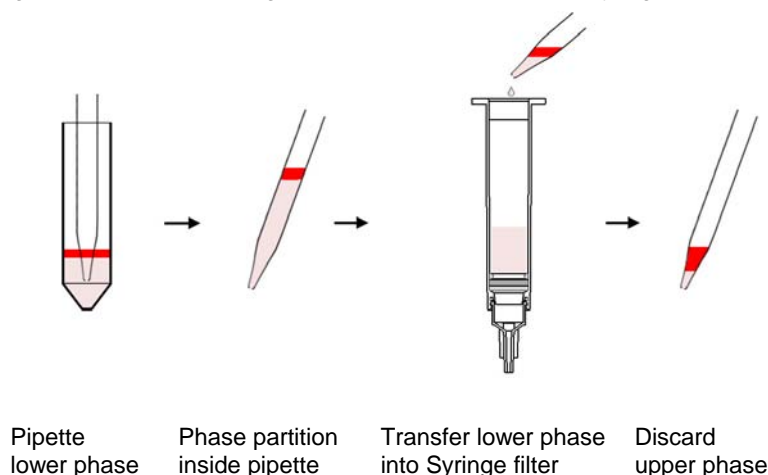
3. Phase partitioning to remove impurities

This protocol uses two successive phase-partitioning steps to purify the genomic DNA. In the first step, the addition of Buffer DV facilitates the separation of the lysate into two phases. The genomic DNA is segregated into the lower phase, while lipids, polysaccharides, metabolites and pigments become segregated into the upper phase. Denatured cellular protein is trapped at the interphase. Following this, the upper phase is discarded and a second phase separation is carried out by the addition of another aliquot of Buffer DV.

4. Removal of the interphase protein by syringe-mediated filtration

As depicted in Figure 1 (below), the lower phase containing the genomic DNA is recovered and transferred to a Midiprep syringe filter. When recovering the lower-phase, a small volume of the upper phase may occasionally also be aspirated. Try to minimize the uptake of any interphase debris. The contaminating upper phase liquid will quickly shift to the top within the pipette and is easily discarded by retaining it within the pipette. The upper phase must not be transferred to the Midiprep syringe filter because it will inhibit binding of DNA to silica membrane. Any interphase debris which is inadvertently aspirated will be recovered by the Midiprep syringe filter.

Figure1: Transfer the genomic DNA-containing lower-phase to the Midiprep Syringe filter



5. Selectively binding of genomic DNA and removal of impurities by washing

Buffer BV is added to the filtrate to establish conditions for binding the genomic DNA to the AxyPrep Midi Column. The AxyPrep Midi Column is washed with Buffer W1 and Buffer W2 to remove residual cell debris and salt, respectively.

6. Elution of genomic DNA

Nuclease-free 1.5 ml microfuge tubes are recommended for the elution and storage of the purified genomic DNA. Store it at -20°C. The AxyPrep DNA Midi column is fitted with a detachable DNA binding module containing silica membranes which bind the genomic DNA. For elution of the purified genomic DNA, this module is detached with a Plastic wrench (provided in the kit) and placed in a standard 1.5 ml microfuge tube.

Precautions

1. Before proceeding with this procedure, make all required preparations to avoid infection by blood-borne viral agents. Please follow local guidelines for working with body fluids and infectious agents.
2. Strictly follow all steps in the protocol, and put all waste in an appropriate Biohazardous Waste container. Autoclave.
3. Buffer VL, Buffer BV and Buffer W1 contain chemical irritants. When working with these buffers, always wear protective clothing such as safety glasses, laboratory coat and gloves. Avoid contacting with eyes or skin. In the event of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and consumables required

- Centrifuge with rotor capable of accommodating 50 ml tubes (either fixed-angle or swinging bucket). $>5,000 \times g$ required
- 50 ml conical centrifuge tubes
- AxyVac Vacuum Manifold (#AP-VAC)
- Vacuum regulator
- Vacuum source capable of -25 - 30 inches Hg (-850 - $1,000$ mbar or -12 - 15 psi)
- Microcentrifuge capable of $12,000 \times g$
- 100% or 95% (denatured) ethanol
- Isopropanol and isobutanol

Preparation before experiment

- 1) Reconstitute Buffer W2: before using the kit, add the volume of ethanol specified on the bottle label to the Buffer W2 concentrate and mix well. 95% denatured ethanol can be used. Make a notation on the bottle label confirming the addition of ethanol for future reference.
- 2) Prepare Buffer DV: Add 2 ml of Buffer DV-A, 125 ml of 100% isopropanol and 75 ml of 100% isobutanol to the 250 ml bottle provided with the kit and mix well.
- 3) Chill Buffer DV to 4°C .

Protocols

Release of DNA

1. Start with 3 ml of whole blood containing anticoagulant in a disposable 50 ml, low-speed centrifuge tube. If the volume of the blood sample is less than 3 ml, make up to 3 ml by supplementing with PBS.
2. Add 6 ml of Buffer VL. Tightly seal the tube with its cap and vortex for 1 minute.
Note: Make sure that the tube is tightly sealed to avoid contamination of the work surface.
3. Add 6 ml of Buffer G-B. Reseal the tube and mix immediately by repeated brisk inversion or shaking.

Phase-partitioning to remove impurities

4. Add 12 ml of Buffer DV pre-chilled to 4°C. Reseal the tube and shake vigorously for several seconds to mix. Centrifuge at $\geq 5,000 \times g$ for 5 minutes.
5. Aspirate off the upper phase (and discard) without disturbing either the interphase or lower phase. Add 12 ml of Buffer DV, pre-chilled to 4°C. Reseal the tube and shake vigorously for several seconds to mix. Centrifuge at $\geq 5,000 \times g$ for 5 minutes.
6. Aspirate off the upper phase (and discard) without disturbing either the interphase or lower phase. Insert the syringe filter barrel into a 50 ml centrifuge tube (supported by a rack). Without disturbing the upper phase or interphase, transfer the lower phase into the barrel of the syringe filter. Insert the plunger into the syringe barrel. Grasp the Syringe filter assembly and with a slow, steady motion push the syringe plunger to filter the lower phase. Collect the filtrate in the 50 ml centrifuge tube.

IMPORTANT: Do not transfer any of the upper phase solution into the syringe filter.

Note: If the lower phase is transferred without any contaminating interphase debris, Step 6 can be omitted.

7. Add 6 ml of Buffer BV to the filtrate and mix well.
8. Attach the AxyVac Vacuum Manifold Base to a vacuum source and regulator. Place the clear vacuum top with the valves and luer-type fittings onto the manifold base. Insert a Column adapter into one of the fittings on the manifold top. Insert the Midiprep column into the Column adapter. Transfer the binding mix from Step 7 to the Midiprep column. Switch on vacuum source adjust to -25-30 inches Hg to draw the solution through the Midiprep column.

Note: The vacuum source must be capable of -25-30 inches Hg. This is equivalent to approximately -850-1,000 mbar or -12-15 psi.

9. Add 8 ml of Buffer W1 and draw the solution through the Midiprep column.
10. Add 9 ml of Buffer W2 along the wall of the tube and draw through the Midiprep column.

Note: Make sure that ethanol has been added into Buffer W2 concentrate.

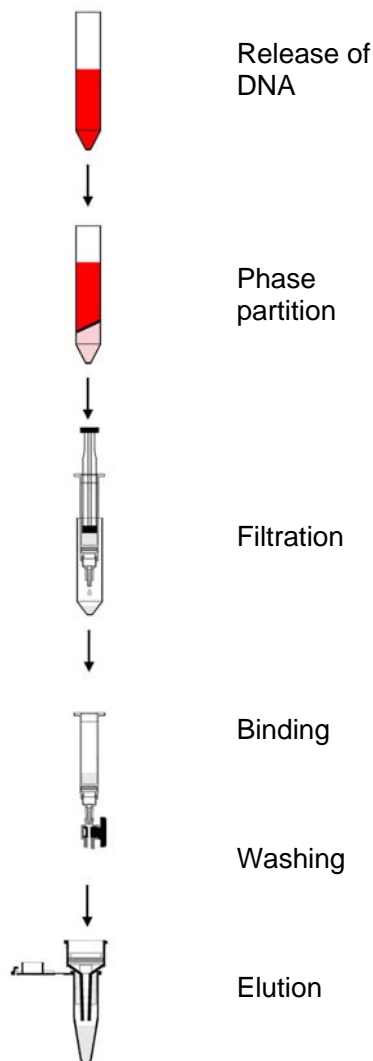
11. Detach the DNA binding module from the Midiprep column with the Plastic wrench provided in the kit. Transfer the DNA binding module into a 1.5 ml microfuge tube, add 0.3 ml of Buffer W2, cover the DNA binding module with Midi-column cap and centrifuge at $12,000 \times g$ for 2 minutes.
12. Transfer the DNA binding module into a clean 1.5 ml microfuge tube. To elute the genomic DNA, add 0.5 ml of deionized water or the Eluent to the centre of the membrane and cover the DNA binding module with Midi-column cap. Let it stand for 2 minutes at room temperature. Centrifuge at $12,000 \times g$ for 1 minute.

Note: Prewarming water or Eluent to 65°C will often improve elution efficiency and yield.

13. **Option:** Eluting again with 0.25 ml of deionized water or Eluent will increase DNA yield. Add 0.25 ml of deionized water or Eluent to the center of membrane. Stand it at room temperature for 1 minute. Centrifuge at $12,000 \times g$ for 1 minute.

Overview

1. Apply 6 ml of Buffer VL in a 50 ml centrifuge tube.
2. Add 3 ml of anticoagulated whole blood. Vortex for 1 minute.
3. Add 6 ml of Buffer G-B.
4. Add 12 ml of Buffer DV pre-chilled at 4°C. Centrifuge at $\geq 5,000 \times g$ for 5 minutes.
5. Discard the upper phase. Add 12 ml of Buffer DV pre-chilled at 4°C. Centrifuge at $\geq 5,000 \times g$ for 5 minutes.
6. Filter the lower phase.
7. Add 6 ml of Buffer BV to the filtrate.
8. Add 8 ml of Buffer W1.
9. Add 9 ml of Buffer W2.
10. Add 0.3 ml Buffer W2.
11. Add 0.5 ml of deionized water or Eluent.



Troubleshooting

1. Low or no yield

- Low WBC content in blood sample
- Inefficient mixing with Buffer VL
- Contamination with upper phase solution resulting in poor binding to silica membrane
- Premature elution. Forgot to add ethanol to Buffer W2 concentrate
- DNA not efficiently eluted

2. Low $A_{260/280}$

- Inefficient lysis with Buffer VL
- Inefficient mixing with Buffer DV
- Excessive number of WBCs in blood sample
- Excessive volume of blood processed

3. Genomic DNA appears to be degraded

Depending upon the extent of degradation, the genomic DNA will either appear as a smear or as a smear trailing in front of a high molecular weight band on an agarose gel. Since no physical measure used during the purification process is sufficient to cause any visually discernible degradation, the most likely source is enzymatic. Enzymatic degradation may result from prolonged or improper storage of the blood sample.

4. Genomic DNA performs poorly in enzymatic reactions

- Salt contamination
- Ethanol contamination

5. Clogged spin-filter

- Elevated WBC content in blood sample
- Excessive interphase debris transferred to spin-filter