AxyPrep Blood Genomic DNA Maxiprep Kit

For the purification of high molecular weight genomic DNA from larger volumes of whole blood

Kit contents, storage and stability

Cat. No.	AP-MX-BL-GDNA-10	AP-MX-BL-GDNA-25
Kit size	10 preps	25 preps
Maxiprep column	10	25
Midiprep syringe filter	10	25
Column adapter	10	25
Buffer VL	120 ml	300 ml
Buffer G-B	120 ml	300 ml
Buffer DV bottle (empty)	1	1
Buffer DV-A	6 ml	15 ml
Buffer BV	120 ml	300 ml
Buffer W1	180 ml	450 ml
Buffer W2 concentrate	2×36 ml	2×150 ml
Eluent	25 ml	60 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. Either 100% or 95% denatured ethanol can be used. Store at room temperature.

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

Introduction

The AxyPrep Blood Genomic DNA Maxiprep Kit is designed for the isolation of up to 250 μg of high molecular weight genomic DNA from up to 5 ml of anticoagulated human or animal whole blood or up to 500 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 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 acid will be adsorbed by the column, while cellular proteins, metabolites and PCR inhibitors are removed. The highly 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 ("leukocyte count") of "normal" human or animal whole blood is defined as a baseline value for this kit. In 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 RBC. 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	Quantity of leukocyte
Normal	$4\text{-}7\times10^6$
Immunosuppression or aplastic anemia	2×10^6
Inflammation	4×10^7
Leukemia	5×10^8

2. Release of genomic DNA

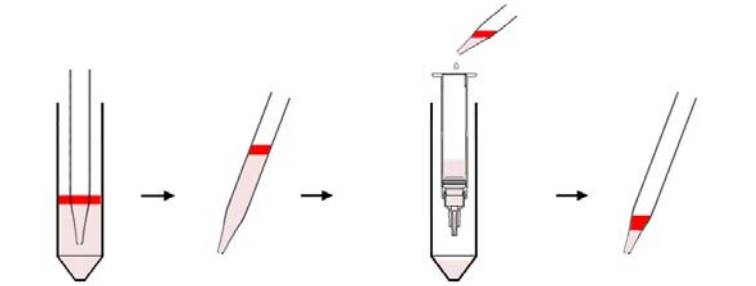
   Buffer VL lyses blood cell, virus and mitochondria directly without the need to separate out RBCs in advance. Buffer VL will also kill any blood-borne viruses present. The RBCs and WBCs are lysed by vigorous vortexing or by passage 6-8× through a 10 ml pipette. The mechanical forces generated in the 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. 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 migrates to the lower phase, while lipid, polysaccharide, 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 generated by the addition of another aliquot of Buffer DV.

4. Removal of the interphase precipitate 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 the Maxiprep column. 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 Maxi Column. The AxyPrep Maxi Column is washed with Buffer W1 and Buffer W2 to remove residual cell debris and salt, respectively.
6. Elution of genomic DNA
   Disposable nuclease-free, 50 ml centrifuge tubes are recommended for the collection and storage of the purified genomic DNA. Store at -20°C. The AxyPrep Maxi column is transferred to a 50 ml centrifuge tube for elution of the purified genomic DNA.

Caution

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 into 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 contact 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 (swinging bucket)≥6,000×g required
- Disposable, 50ml centrifuge tubes
- AxyVac Vacuum Manifold (#AP-VAC)
- Vacuum regulator
- Vacuum source capable of –25-30 inches Hg
- Nuclease-free disposable pipettes
- 100% or 95% (denatured) ethanol
- Isopropanol and isobutanol

Preparation before experiment

1) Before using this 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 4 ml of Buffer DV-A, 250 ml of isopropanol and 150 ml of isobutanol to 500 ml bottle provided with kit and mix well.
3) Chill Buffer DV to 4°C.

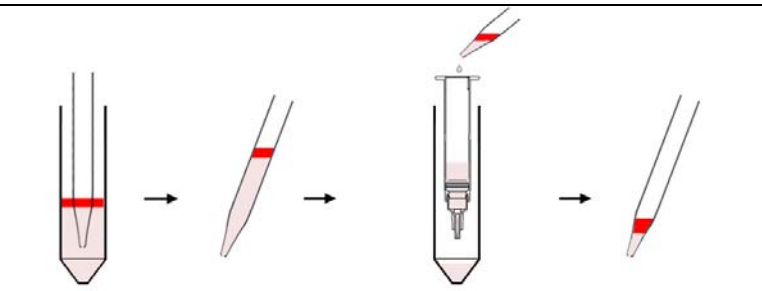
Protocols

Release of DNA

1. Start with 5 ml of whole blood containing anticoagulant in a disposable 50 ml, low-speed centrifuge tube. If the volume of blood is less than 5 ml, supplement with PBS.
2. Add 10 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 leakage and contamination of the work surface.
3. Add 10 ml of Buffer G-B. Reseal the tube and mix immediately by repeated brisk inversion or shaking.

Phase partitioning to remove impurities

4. Add 20 ml of Buffer DV pre-chilled at 4°C. Reseal the tube and shake vigorously for several seconds to mix. Centrifuge at ≥5,000×g for 5 minutes.
5. Aspirate off the upper phase (and discard) without disturbing either the interphase or the lower phase. Add 20 ml of Buffer DV (pre-chilled to 4°C). Reseal the tube and shake vigorously for several seconds. Centrifuge at ≥5,000×g for 5 minutes.
6. Insert the Midiprep 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 Midiprep syringe filter. Insert the plunger into the syringe barrel. Grasp the Midiprep 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 upper phase solution into the Midiprep syringe filter.



7. Add 10 ml of Buffer BV to the filtrate and mix well.
8. Attach the AxyVac Vacuum Manifold Base to a vacuum source and regulator. Position the clear vacuum top with valves and luer-type fittings onto the manifold base. Insert a Column adapter into one of the fittings on the manifold top. Insert the Maxiprep column into the Column adapter. Transfer the binding mix from Step 7 to the Maxiprep column. Switch on the vacuum source and adjust to -25-30 inches Hg.

   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. After all of the binding solution in Step 8 has passed through the Maxiprep column, add 15 ml of Buffer W1 and draw the solution through the Maxiprep column.
10. Add 20 ml of Buffer W2 along the wall of the Maxiprep column. Apply vacuum until no liquid remains in the column. Turn the vacuum off.

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

11. Transfer the Maxiprep column into a 50 ml centrifuge tube and centrifuge at ⩾6,000×g for 5 minutes.

    Note: Keep the Column adapter on the Vacuum manifold for the use in the Step 12.

12. Insert the Maxiprep column back into the Column adapter on the manifold top. Continue to apply vacuum on for additional 5 minutes to remove residual Buffer W2.
13. Transfer the Maxiprep column into a new 50 ml centrifuge tube. To elute the genomic DNA, add 1.5 ml of deionized water or Eluent to the center of membrane. Allow it to stand it at room temperature for 5 minutes. Centrifuge at ⩾6,000×g for 5 minutes.

    Note: Pre-warming water or Eluent at 65°C will often improve elution efficiency.

14. **Option:** Eluting again with 0.75 ml of water or Eluent will increase about 30% of DNA yield. Add 0.75 ml of deionized water or Eluent to the center of membrane. Stand it at room temperature for 1 minute. Centrifuge at ⩾6,000×g for 5 minutes.

Overview

1. Apply 10 ml of Buffer VL in a 50 ml centrifuge tube.
2. Add 5 ml of anticoagulant whole blood. Vortex for 1 minute.

Release of DNA

3. Add 10 ml of Buffer G-B.
4. Add 20 ml of Buffer DV pre-chilled at 4℃. Centrifuge at ⩾5,000×g for 5 minute.
5. Discard the upper-phase. Add 20 ml of Buffer DV pre-chilled at 4°C. Centrifuge at ⩾5,000×g for 5 minutes.

Phase Partition

6. Filter the lower phase.

Filtration

7. Add 10 ml of Buffer BV to the filtrate.

Binding

8. Add 15 ml of Buffer W1.
9. Add 20 ml of Buffer W2.

Washing

10. Add 1.5 ml of deionized water or Eluent.

Elution

Troubleshooting

1. Low or no yield

- Low WBC content in blood sample
- Inefficient mixing with 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
- Excesive volume of blood processed

3. Genomic DNA appears to be degraded

Depending upon the completeness 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 discernable 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