AxyPrep Blood Genomic DNA Miniprep Kit

For the purification of genomic DNA from whole blood

Kit contents, storage and stability

Cat. No.	AP-MN-BL-GDNA-50	AP-MN-BL-GDNA-250
Kit size	50 preps	250 preps
Miniprep column	50	250
2 ml Microfuge tube	50	250
1.5 ml Microfuge tube	50	250
Buffer AP1	30 ml	150 ml
Buffer AP2	6 ml	30 ml
Buffer W1A concentrate	24 ml	120 ml
Buffer W2 concentrate	24 ml	2×72 ml
Buffer TE	11 ml	60 ml
Protocol manual	1	1

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

Buffer AP1: Cell lysis buffer. Store at room temperature.

Buffer AP2: Protein-depleting buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. Store at room temperature. Add amount of ethanol specified on the bottle label and store at room temperature. Either 100% or 95% denatured ethanol can be used.

Buffer W2 concentrate: Desalting buffer. Add amount of ethanol specified and store at room temperature. Either 100% or 95% denatured ethanol can be used.

Buffer TE: 5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5. Store at room temperature.

Introduction:

This method is based on the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and heme/protein precipitation buffer (Buffer AP1) coupled with the selective adsorption of the genomic DNA to a special AxyPrep column. The purified genomic DNA is eluted in a low-salt Tris buffer containing 0.5 mM EDTA, which enhances DNA solubility and helps to protect the high molecular weight DNA against subsequent nuclease degradation. Blood genomic DNA is directly isolated from the white blood cell (WBC) component of whole blood, without the need to remove the red blood cells (RBCs) in advance. This kit can also be used to extract DNA from dried blood. Viral DNA, viral RNA and mitochondrial DNA are co-purified with the genomic DNA by this procedure. Buffer AP1 will efficiently destroy any bacteria and virus present in the blood sample, thereby reducing the potential risk of these infectious agents.

Caution

Buffer AP1 and Buffer W1A contain chemical irritants. When working with these buffers, always wear suitable protective clothing such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and additional consumables required

- Microcentrifuge capable of 12,000×g
- Heated water bath
- 100% or 95% (denatured) ethanol

Preparation before experiment

Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W1A and Buffer W2 concentrates and mix well. Either 100% or 95% (denatured) ethanol can be used.

Protocols

The AxyPrep protocol is suitable for purifying up to 12 μg of genomic DNA from 250 μl of anti-coagulated whole blood. The Miniprep column has a maximum binding capacity of approximately 25 μg. If more genomic DNA is required, process 0.5 ml of whole blood in the following manner. To extract DNA from 0.5 ml of blood, divide the sample into 2×250 μl aliquots and prepare extracts by following Steps 1-4 in two separate Microfuge tubes. Combine the supernatant obtained in step 4 into one Miniprep column to consolidate the genomic DNA and increase the yield. Elute the purified genomic DNA in 100-200 μl of Buffer TE.

For maintaining the integrity and reactivity of the genomic DNA, particularly in PCR, the purified genomic DNA should be eluted and stored in low-salt Tris buffer containing 0.5-1 mM EDTA (provided).

We recommend the use of nucleic acid-free and nuclease-free plasticware.

1. Add 500 μl of Buffer AP1 to a 1.5 ml microfuge tube.
2. Add 200-250 μl of anti-coagulated whole blood. Close the cap of the Microfuge tube and mix by vortexing at top speed for 10 seconds.

   Note: Vortexing is required for complete release of the genomic DNA. Although vortexing will result in limited shearing of the genomic DNA, it will have no effect upon the performance of the genomic DNA in applications which require high molecular DNA.

   Note: To extract genomic DNA from clotted or dried blood, place the sample in a mortar (ambient temperature) and add 200 μl of 20 mM Tris, 10 mM EDTA, pH 8.5. Grind rapidly for 30 seconds to disperse the sample. Add 500 μl of Buffer AP1 pre-heated to 50°C and grind briefly or pipette to dissolve the sample. Transfer

the sample to a 1.5 ml Microfuge tube with a transfer pipette or other device. Vortex for 10 seconds to further dissolve the dried or clotted blood.

Note: If blood volume is <200 μl, add deionized water or PBS to 200 μl.

Note: If using avian blood or amphibian blood, combine <10 μl of blood with 200 μl of PBS.

3. Add 100 μl of Buffer AP2 and mix by vortexing at top speed for 10 seconds.

4. Centrifuge at 12,000×g for 10 minutes at ambient temperature to pellet cellular debris.

5. Place a Miniprep column into a 2 ml Microfuge tube. Pipette the clarified supernatant obtained from step 4 into the Miniprep column. Centrifuge at 12,000×g for 1 minute.

6. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Pipette 700 μl of Buffer W1A into the Miniprep column and allow to stand at room temperature for 2 minutes. Centrifuge at 12,000×g for 1 minute.

   Note: Be sure that ethanol has been added to the W1A concentrate.

   Note: If any liquid remains in the Miniprep column after centrifugation, extend the centrifuge time or increase the g-force.

7. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Add 800 μl of Buffer W2 to the Miniprep column and centrifuge at 12,000×g for 1 minute.

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

8. **Optional Step:** Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Add 500 μl of Buffer W2 to the Miniprep column and centrifuge at 12,000×g for 1 minute.

   Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

9. Discard the filtrate from the 2 ml Microfuge tube. Place the Miniprep column back into the 2 ml Microfuge tube. Centrifuge at 12,000×g for 1 minute.

10. Place the Miniprep column into a 1.5 ml Microfuge tube (provided). Add 80-200 μl of Buffer TE. Allow to stand at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the genomic DNA.

    Note: Pre-warming Buffer TE at 65°C will generally improve elution efficiently.

Overview

Troubleshooting

1. Low or no yield

- Low WBC content in blood sample
- Inefficient lysis with Buffer AP1
- Inefficient mixing with Buffer AP2
- DNA not efficiently eluted
- Blood coagulation

2. Low $A_{260/280}$

- Inefficient lysis with Buffer AP1
- Inefficient mixing with Buffer AP2
- Buffers AP1 and AP2 used in the wrong order
- Blood has been stored at room temperature for extended periods

3. Genomic DNA appears to be degraded

Blood has been stored at room temperature for extended periods.

Depending upon the completeness of degradation, the genomic DNA will either appear as a diffuse 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

- Low DNA concentration
- Salt contamination
- Ethanol contamination

5. Clogged spin-filter

- Elevated WBC content in blood sample
- Inefficient lysis with Buffer AP1
- Inefficient mixing with Buffer AP2
- Blood was insufficiently mixed after phlebotomy, resulting in coagulation
- Precipitates have formed in blood that has been stored either frozen or at room temperature for extended periods

6. Clogged Miniprep column membrane

- Elevated WBC content in blood sample
- Inefficient lysis with Buffer AP1
- Inefficient mixing with Buffer AP2