AxyPrep-96 Blood Genomic DNA Kit

**For the purification of genomic DNA
from whole blood in a 96-well format**

Kit contents, storage and stability

Cat. No.	AP-96-BL-GDNA-4	AP-96-BL-GDNA-12
Kit size	4×96	12×96
Round-well block	4	12
96-well 1.6 ml growblock	8	24
96-well DNA plate	4	12
Round-well silicone mat	4	12
BF-400 breathable film	20	60
Proteinase K	120 mg (9 ml)	360 mg (27 ml)
PK Buffer	10 ml	30 ml
Buffer BL	92 ml	250 ml
Buffer W1B concentrate	84 ml	2×122 ml
Buffer W2 concentrate	3×72 ml	-
10×Buffer W2 concentrate	-	75 ml
Buffer W2 bottle (empty)		1
Eluent B	92 ml	250 ml
Protocol Manual	1	1

Except for Proteinase K, all buffers are stable for a period of at least 12 months when stored under ambient conditions. Please avoid exposure to direct sunlight and extremes in temperature. After reconstitution, Proteinase K is stable for 2 months when stored at 4℃. To preserve Proteinase K activity, the lyophilized Proteinase K is resuspended in PK Buffer, which containing a high concentration of ammonium sulfate. On occasion, a precipitate may form. If this occurs, resuspend it by vortexing or pipetting before use. The Proteinase K activity is unaffected.

Proteinase K: Lyophilized Proteinase K is stable for up to 6 months after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 4°C is recommended. After reconstitution, the Proteinase K is stable for 2 months when stored at 2~8°C. Storing the Proteinase K stock solution at room temperature for prolonged periods of time should be avoided.

PK Buffer: Used to dissolve Proteinase K. Store at room temperature.

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

Buffer W1B concentrate: Wash buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

10×Buffer W2 Concentrate (only in the 12×96 kit): Before use of the kit, add 15 ml of 10× Buffer W2 concentrate, 135 ml of deionized water, 350 ml of ethanol to the empty 500 ml bottle supplied with the kit. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Eluent B: 7.5 mM Tri-HCl, pH 8.5, 0.3 mM EDTA. Store at room temperature.

Introduction:

The isolation of genomic DNA from blood by this kit is based upon the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and protein removal buffer in combination with Proteinase K digestion. The genomic DNA is then selectively adsorbed to a special AxyPrep 96-well DNA plate for further purification and desalting. Genomic DNA is directly isolated from the white blood cell (WBC) component of whole blood, without the need to first remove the red blood cells (RBCs). This method requires no phenol/chloroform extraction or ethanol precipitation. The purified genomic DNA is eluted in a low-salt Tris buffer or water and is ready for immediate use in downstream applications such as PCR or Southern blotting. Genomic DNA purified by this method is free of protein, nucleases, and other cellular contaminants and exhibits a size range of approximately 30-50 kb.

This kit can also be used to extract DNA from frozen whole blood, plasma, serum, bone marrow, other body fluids, lymphocytes and cultured cells. Viral and mitochondrial DNA will also be copurified with genomic DNA by this procedure.

Caution:

Buffers BL and Buffer W1B contain chemical irritants. When working with these buffers, always wear protective clothing such as safety glasses, gloves and laboratory coat. 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 consumables required

Adjustable temperature water bath
Centrifuge with swinging bucket rotor and plate carriers (6,000 rpm required)
AxyVac Vacuum Manifold (Cat. No. AP-VAC) or other vacuum manifold
Vacuum regulator
Vacuum source (capable of –25-30 inches Hg)
95-100% ethanol
8- or 12-channel pipette

Preparation before experiment

1) Adjust water bath to 70°C.
2) Before the use of the kit, add ethanol to both the Buffer W1B concentrate and the Buffer W2 concentrate as much as specified on the bottle labels. Mix well.
3) Resuspend Proteinase K in PK Buffer.
4) Check Buffer BL for precipitation before use. If precipitation occurs, incubate at 70°C to dissolve the precipitate.
5) Preparation of Buffer W2 (12×96 kit). Add the following to the empty bottle labeled "Buffer W2 Bottle" provided in the kit:
   - 15 ml 10× Buffer W2 concentrate
   - 135 ml deionized H_2O
   - 350 ml ethanol (100% or 95% denatured)

Protocol

1. Pipette 20 µl of Proteinase K solution into each well of the Round-well block.
2. Add either 200 µl of whole blood, plasma, serum, or body fluids to each well of the Round-well block, or up to 5×10^6 lymphocytes or cultured cells suspended in 200 µl of PBS per well. Deposit the blood (or other) samples into the wells without wetting the rims.
   Note: If the blood sample volume is less than 200 µl, supplement with PBS.
   Note: If using avian blood or amphibian blood, combine <10 µl of blood with 200 µl of PBS.
   Note: If RNA-free genomic DNA is required, add 20 µl of DNase-free RNase A (20 mg/ml) to the sample before the addition of Buffer BL in Step 3 (below).

3. Add 200 µl Buffer BL to each sample, taking care not to wet the rims of the wells. Seal the wells using a Round-well silicone mat.
   Note: Be sure that the wells are properly sealed to avoid leaks during shaking.

4. Mix thoroughly by shaking vigorously for 30 seconds.
   IMPORTANT: For efficient lysis, it is essential that the samples and Buffer BL are mixed immediately and thoroughly to yield a homogeneous solution. Hold the sealed Round-well Block with both hands and shake up and down vigorously. Inversion or vortexing will not provide sufficient force.

5. Centrifuge briefly at 3,000 rpm to collect any solution from the Round-well silicone mat. Allow the centrifuge to reach 3,000 rpm, then stop.
   Note: Be sure to use a counterbalance when processing samples in a single Round-well Block.

6. Incubate at 70°C for at least 10 minutes in an incubator or oven.
   Note: Place a weight on the Round-well silicone mat, to avoid the Round-well silicone mat separate from Round-well block.
   Note: Longer incubation times have no effect on the quality of the purified DNA.

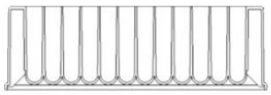
7. Centrifuge briefly at 3,000 rpm to collect any lysate from the Round-well silicone mat. Allow centrifuge to reach 3,000 rpm, then stop.
8. Remove the Round-well silicone mat and add 200 µl ethanol (96 – 100%) to each well.
9. Seal the wells using the Round-well silicone mat. Shake vigorously for 15 seconds.
10. Centrifuge briefly at 3,000 rpm to collect any solution from the Round-well silicone mat. Allow the centrifuge to reach 3,000 rpm, then stop.
11. Place a 96-well DNA plate onto a 1.6 ml growblock. Remove the Round-well silicone mat from the Round-well block. Transfer the lysates to the wells of the DNA plate. Seal the top of the 96-well DNA plate with a piece of clear BF-400 breathable film. Centrifuge at 6,000 rpm for 4 minutes.
12. Discard the filtrate from the wells of the 1.6 ml growblock. Place the DNA plate back onto the 1.6 ml growblock. Add 500 µl of Buffer W1B to the 96-well DNA plate. Seal the top of the 96-well DNA plate with a piece of clear BF-400 breathable film. Centrifuge at 6,000 rpm for 4 minutes.
    Note: Make sure that ethanol has been added to the Buffer W1B concentrate.

13. Discard the filtrate from the wells of the 1.6 ml growblock. Place the DNA plate back onto the 1.6 ml growblock. Add 850 µl of Buffer W2 along the walls of the DNA plate wells to wash off any residual Buffer W1B. Seal the top of the 96-well DNA plate with a piece of clear BF-400 breathable film. Centrifuge at 6,000 rpm for 4 minutes.
    Note: Make sure that ethanol has been added into Buffer W2 concentrate.

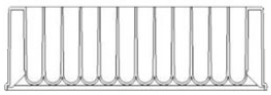
14. Discard the filtrate from the wells of the 96-well 1.6 ml growblock. Place the DNA plate onto the 1.6 ml growblock, add 400 µl of Buffer W2 to the DNA plate. Seal the top of the 96-well DNA plate with a piece of clear BF-400 breathable film. Centrifuge at 6,000 rpm for 15 minutes.
15. Place the DNA plate onto a new 1.6 ml growblock. To elute the purified genomic DNA, Add 100-200 µl of Eluent B or deionized water, and let it stand for 2 minutes at room temperature. Seal the top of the DNA plate with a piece of clear BF-400 breathable film. Centrifuge at 6,000 rpm for 4 minutes.

Overview

Add 20 µl of Proteinase K
Add 200 µl of samples



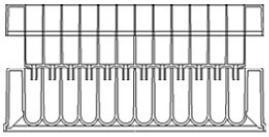
Add 200 µl of Buffer BL
Incubate at 70°C for 10 min



Lysis

Add 200 µl of ethanol
Transfer the lysate to the 96-well DNA plate

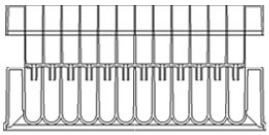
Add 500 µl of Buffer W1B
Add 850 µl of Buffer W2
Repeat wash with Buffer W2



Binding

Washing

Add 100-200 µl of Eluent B or deionized water



Elution

Troubleshooting

Clogged wells in the 96-well DNA plate

- Coagulation of sample. Remove clots and repeat.
- Precipitates have formed in blood that has been stored either frozen or at room temperature for extended periods. Use fresh blood.
- Elevated WBC content or buffy coat used. Dilute sample with PBS.
- Insufficient lysis due to inadequate mixing. Mix samples and Buffer BL immediately and thoroughly.
- Insufficient lysis due to reduced Proteinase K activity. Use new Proteinase K stock.

Colored residues (heme) remain on the 96-well DNA plate after washing

- Inefficient cell lysis due to insufficient mixing of the samples with Buffer BL.
- Inefficient lysis due to reduced proteinase activity. Use new proteinase K stock.
- Buffer W1B or Buffer W2 prepared incorrectly. Ensure that Buffer W1B or Buffer W2 concentrates were diluted with the correct amounts of ethanol.

Little or no DNA in the eluate

- Low WBC content in blood sample.
- No ethanol added to the lysate before loading onto 96 plate. Repeat the purification procedure with the new samples.
- Inefficient cell lysis due to insufficient mixing of the sample with Buffer BL. Mix the samples and Buffer BL immediately and thoroughly.
- Inefficient cell lysis or protein degradation due to insufficient incubation time or temperature.
- Inefficient lysis due to reduced Protease activity. Repeat the purification procedure with new proteinase K.
- 96-well DNA plate not incubated at room temperature for 1 minute prior to elution.
- DNA not eluted efficiently. To increase elution efficiency, incubate Eluent B or deionized water at 65°C before loading onto membrane.

Low $A_{260/280}$ ratio

- Inefficient cell lysis due to insufficient mixing of the sample with Buffer BL samples.
- Inefficient cell lysis or protein digestion due to insufficient incubation time or temperature.
- Inefficient lysis due to reduced proteinase K activity. Use new proteinase K samples.
- Buffer W1B or Buffer W2 prepared incorrectly were diluted with the correct amount of ethanol.

Genomic DNA performs poorly in enzymatic reactions

- Not enough DNA in sample Check “Little or no DNA in the eluate” for possible reasons.
- Purified DNA contaminated with inhibitory substances. Check “$A_{260/280}$”.
- DNA degraded. Run sample on an analytical gel to assess intactness.
- Excessive DNA in the PCR. Repeat the PCR, using less DNA template.
- Residual Buffer W2 in the eluate.