FastPure Blood/Cell/Tissue/Bacteria DNA Isolation Mini Kit

DC112

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

This kit is suitable for extracting genomic DNA from ≤200 µl of fresh or frozen anticoagulated whole blood, <25 mg of animal tissues, <5 × 10^6 of cultured cells and <3 × 10^9 cultured bacterial samples. The kit is based on silica gel column purification technology that eliminates the need for extraction using phenol/chloroform organic solvents or time-consuming alcohol precipitation step. With this kit, RNA, proteins, lipids and other inhibitory impurities can be removed at the greatest extent. The DNA obtained can be directly used in PCR, qPCR, enzyme digestion and virus detection.

02/Components

Components	DC112-01 (50 rxns)	DC112-02 (200 rxns)
Buffer ACL	12 ml	48 ml
Proteinase K	1 ml	4 ml
Buffer BCL	12 ml	48 ml
Buffer WA	15 ml	60 ml
Buffer WB	20 ml	2 × 40 ml
Elution Buffer	20 ml	80 ml
FastPure gDNA Mini Columns II	50	2 × 100
Collection Tubes 2 ml	50	2 × 100

Buffer ACL: Sample lysis.

Proteinase K: Perform enzymatic lysis of samples.

Buffer BCL: Perform lysis of samples and provide the binding environment.

Buffer WA: Remove residual protein contamination.

Buffer WB: Remove salt ions.

Elution Buffer: Elute the bound DNA.

FastPure gDNA Mini Columns II: Genomic DNA adsorption column.

Collection Tubes 2 ml: Filtrate collection tubes.

03/Storage

Store at 15 ~ 25°C and transport at room temperature.

If ambient temperatures often exceed 25°C, we suggest storing Proteinase K at 2 ~ 8°C.

04/Applications

Fresh or frozen anticoagulated whole blood without nuclei (≤200 µl).

Fresh or frozen anticoagulated whole blood with nuclei (5 - 20 µl).

Cultured cells (<5 × 10^6).

Animal tissues (<25 mg).

Bacteria (<3 × 10^9).

05/Self-prepared Materials

PBS, RNase A (Vazyme #DE111) (optional), Lysozyme (Vazyme #DE103) (for Gram-positive bacteria extraction), absolute ethanol, sterilized 1.5 ml centrifuge tube, water bath, etc.

06/Notes

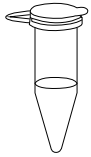
For research use only. Not for use in diagnostic procedures.

1. Please add a specified amount of absolute ethanol to Buffer WA and Buffer WB according to the label before use.

Reagent Name	DC112-01 WA	DC112-01 WB	DC112-02 WA	DC112-02 WB
Volume of absolute ethanol (ml)	20	80	80	160

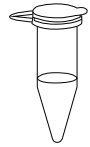
2. Avoid repeated freezing and thawing of samples, otherwise it will lead to the degradation of the extracted genomic DNA and reduce the extraction efficiency.
3. Check if there is any precipitation in Buffer ACL, Buffer BCL and Buffer WA before use. If precipitates have formed, they can be re-dissolved in a 37℃ water bath and mixed well before use.
4. All procedures must be carried out at room temperature (15 ~ 25℃).

07/Mechanism & Workflow



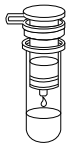
Sample processing

- **Blood samples:** Input 200 µl blood (If the volume of the blood is not enough, make up with Buffer ACL) to the tube. Add 20 µl Proteinase K and 200 µl Buffer BCL to the sample in sequence, mix thoroughly by vortexing, and incubate in a 70℃ water bath for 10 min.
- **Cell samples:** Add 200 µl PBS, 20 µl Proteinase K, and 200 µl Buffer BCL to the sample in sequence, mix thoroughly by vortexing, and incubate at 56℃ for 10 min.
- **Tissue samples:** Add 200 µl Buffer ACL and 20 µl Proteinase K to the sample in sequence, vortex and mix, and incubate at 56℃ until complete enzymolysis; then add 200 µl Buffer BCL, mix thoroughly by vortexing.
- **Bacterial samples:** (Gram-negative bacteria) Add 200 µl Buffer ACL, 20 µl Proteinase K, and 200 µl of Buffer BCL to the sample in sequence, mix thoroughly by vortexing, and incubate at 56℃ for 10 min. (Gram-positive bacteria) Add 180 µl Lysozyme to the sample, incubate at 37℃ for 30 min, then add 20 µl Proteinase K and 200 µl Buffer BCL in sequence, mix thoroughly by vortexing, incubate at 56℃ for 10 min.



Adjust the binding environment: Add 150 µl absolute ethanol and mix by vortexing.

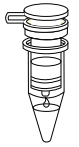
Bind genome DNA: Transfer the mixture to an adsorption column and centrifuge at 12,000 rpm (13,400 × g) for 1 min.



Removal of impurities such as protein: Add 500 µl Buffer WA to the adsorption column and centrifuge at 12,000 rpm (13,400 × g) for 1 min. Discard the filtrate.

Removal of salt ions: Add 600 µl Buffer WB and centrifuge at 12,000 rpm (13,400 × g) for 1 min (twice). Discard the filtrate.

Removal of ethanol: Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min, then open the lid and air-dry at room temperature for 2 - 5 min.



Elution: Add 50 - 200 µl Elution Buffer, incubate at room temperature for 2 - 5 min, then centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

08-1/Sample Processing

◇ **Blood samples**

1. Place blood sample (≤200 µl) in a 1.5 ml centrifuge tube. Add Buffer ACL to make up to 200 µl, then shake to mix.
   ▲ For samples of anticoagulated whole blood with nucleated erythrocyte taken from poultry, birds and amphibians, the sample volume should be 5 - 20 µl.
   ▲ If the volume of blood is >200 µl, please use Vazyme #DC111 (applicable range: 0.1 - 1 ml).
2. Sequentially add 20 µl Proteinase K and 200 µl Buffer BCL and shake to mix well.
3. Incubate the mixture at 70℃ for 10 min, mix by inverting several times during the incubation. Ensure that there are no granular precipitates.
4. Proceed to 08-2/Column Purification.

◇ **Cell samples**

1. The total number of cells must not exceed 5×10^6. Collect the cells by centrifuging at 300 × g for 5 min, discarding the supernatant.
   ▲ Adherent cells: The cells can be peeled off with a cell scraper or a clean pipette tip, or digest the cells with trypsin, and then centrifuge to collect cells.
2. Add 200 µl PBS and 20 µl Proteinase K to the sample in sequence, and mix thoroughly by vortexing.
   ▲ (Optional) If RNA residues significantly affect subsequent experiments, add 4 µl RNase A (100 mg/ml) (Vazyme #DE111) in Step 2. Mix by inverting, and place at room temperature for 2 - 5 min.
3. Add 200 µl Buffer BCL to the mixture, mix thoroughly by vortexing, and incubate at 56℃ for 10 min. During this period, mix by inverting several times. There may be genomic agglomerates in the solution, which is normal.
4. Proceed to 08-2/Column Purification.

◇ **Tissue samples**

1. Place <25 mg of chopped or ground tissues (For spleen and kidney, the amount should be less than 10 mg) into a 1.5 ml centrifuge tube. Then sequentially add 200 µl Buffer ACL and 20 µl Proteinase K, and shake to mix.
   ▲ (Optional) If the RNA residues significantly affect subsequent experiments, add 4 µl RNase A (100 mg/ml) (Vazyme #DE111) in Step 2. Mix by inverting, and incubate at room temperature for 2 - 5 min.
   ▲ Excessive amount of samples will result in a reduction in DNA yield and purity. Samples such as liver, spleen and kidney are rich in DNA, and the samples shall be less than 10 mg in weight. For tissues with low DNA contents such as muscle and skin, samples can be increased to 20 - 50 mg in weight while proportionally increasing the amount of Buffer ACL, Proteinase K, Buffer BCL and absolute ethanol.

2. Place in a water bath at 56℃ to complete enzymatic lysis. Mix by inverting several times during this period. Ensure that there are no granular precipitates.
   ▲ Mix by inversion to promote the lysis process. Chop the tissues into pieces to shorten the digestion time. Digestion time is dependent on the sample type and homogenization results. In general, 0.5 - 3 h is required for tissue samples, 6 - 8 h or overnight digestion is required for mouse tails.
   ▲ If the tissue sample is ground into powder using liquid nitrogen, it can be extracted directly using the simple operating procedure for tissue samples.
3. Add 200 µl Buffer BCL to the lysate, and mix thoroughly by vortexing.
4. Proceed to 08-2/Column Purification.

● **Simple operating procedure for tissue samples (This procedure can be used for tissue samples that are sufficiently ground into powder using liquid nitrogen)**

1. Place <25 mg ground tissue (For spleen and kidney, the amount should be less than 10 mg) in a 1.5 ml centrifuge tube. Then sequentially add 200 µl Buffer ACL and 20 µl Proteinase K, and vortex until there are no granular precipitates.
   ▲ (Optional) If RNA residues significantly affect subsequent experiments, 4 µl RNase A (100 mg/ml) (Vazyme #DE111) can be added in Step 1. Mix by inverting and incubate at room temperature for 2 - 5 min.
2. Add 200 µl Buffer BCL to the mixture, and mix thoroughly by vortexing.
3. Proceed to 08-2/Column Purification.

◇ **Bacteria samples**

Gram-negative bacteria

1. Centrifuge 1 - 5 ml of bacterial culture solution (<3.0 × 10^9 bacteria) at 10,000 rpm (11,500 × g) for 1 min, discarding the culture solution
   ▲ The bacteria number can be measured by spectrophotometer. When OD_{600} = 1.0, the bacteria number is about 1.5 × 10^9/ml.
2. Add 200 µl Buffer ACL, 20 µl Proteinase K, and 200 µl Buffer BCL to the sample in sequence, and mix thoroughly by vortexing.
3. Incubate the mixture at 56℃ for 10 min, mix by inverting several times during the incubation. Ensure that there are no granular precipitates.
   ▲ (Optional) If RNA residues significantly affect subsequent experiments, 4 µl RNase A (100 mg/ml) (Vazyme #DE111) can be added after Step 3. Then mix by inverting and place at room temperature for 2 - 5 min.
4. Proceed to 08-2/Column Purification.

Gram-positive bacteria

1. Centrifuge 1 - 5 ml of bacterial culture solution (<3.0 × 10^9 bacteria) at 10,000 rpm (11,500 × g) for 1 min, discarding the culture solution.
   ▲ The bacteria number can be measured by spectrophotometer. When OD_{600} = 1.0, the bacteria number is about 1.5 × 10^9/ml.

2. Add 180 µl Lysozyme (20 mg/ml) (Vazyme #DE103), shake to re-suspend the bacteria, and incubate at 37℃ for 30 min.

   ▲ The cell walls of most bacteria will break down sufficiently after 30 min, but certain bacteria with thicker cell walls (such as *Staphylococcus aureus*) need to be treated for 1 - 2 h to completely break down cell walls. Please adjust the incubation time according to the types of bacteria.

   ▲ Gram-positive cocci with particularly thick cell walls can be extracted using the complex bacteria extraction procedure.

3. Add 20 µl Proteinase K and 200 µl Buffer BCL to the lysate in sequence, and mix thoroughly by vortexing.

   ▲ (Optional) If the RNA residues significantly affect subsequent experiments, 4 µl RNase A (100 mg/ml) (Vazyme #DE111) can be added in Step 3, then mix by inverting and place at room temperature for 2 - 5 min.

4. Incubate the mixture at 56℃ for 10 min, and mix by inverting several times during the incubation. Ensure that there are no granular precipitates.

5. Proceed to 08-2/Column Purification.

● Complex bacteria (Gram-positive cocci with particularly thick cell walls)

1. Centrifuge 1 - 5 ml of bacterial culture solution (<3.0 × 10^9 bacteria) at 10,000 rpm (11,500 × g) for 1 min, discarding the culture solution.

   ▲ The bacteria number can be measured by spectrophotometer. When OD_{600} = 1.0, the bacteria number is about 1.5 × 10^9/ml.

2. Add 1 ml 70% ethanol and shake to re-suspend the bacteria, then place in an ice bath for 20 min. Centrifuge at 10,000 rpm (11,500 × g) for 1 min, discarding the supernatant.

3. Add 180 µl Lysozyme (Vazyme #DE103), shake to resuspend the bacteria, and incubate at 37℃ until the cell walls are broken down. Centrifuge at 10,000 rpm (11,500 × g) for 1 min, discarding the supernatant.

   ▲ The cell walls of most bacteria will break down sufficiently after incubating for 30 min, but certain Gram-positive cocci with particularly thick cell walls needs to be treated for 3 h to completely break down their walls. Please adjust the incubation time according to the types of bacteria.

4. Add 200 µl Buffer ACL and 20 µl Proteinase K in sequence, shake to resuspend the bacteria to the mixture, and incubate at 56℃ for 30 min.

5. Add 200 µl Buffer BCL to the lysate, and mix thoroughly by vortexing.

   ▲ (Optional) If RNA residues significantly affect subsequent experiments, add 4 µl RNase A (100 mg/ml) (Vazyme #DE111) in Step 2. Mix by inverting, and incubate at room temperature for 2 - 5 min.

6. Incubate at 70℃ for 10 min.

7. Proceed to 08-2/Column Purification.

08-2/Column Purification

1. Add 150 µl absolute ethanol to the sample, and mix thoroughly by vortexing. Flocculent precipitate may appear, then centrifuge briefly to collect the liquid.
2. Place the FastPure gDNA Mini Columns II in Collection Tubes 2 ml. Transfer the mixture above (including the precipitate) to the adsorption column. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
3. Discard the flow-through and place the adsorption column in collection tubes. Add 500 µl Buffer WA along the tube wall (Check whether absolute ethanol has been added) to the adsorption column, and centrifuge at 12,000 rpm (13,400 × g) for 1 min.
4. Discard the flow-through and place the adsorption column in collection tubes. Add 600 µl Buffer WB along the tube wall (Check whether absolute ethanol has been added), and centrifuge at 12,000 rpm (13,400 × g) for 1 min, discarding the flow-through.
5. Repeat Step 4.
6. Place the adsorption column in the collection tube. Centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
   ▲ After centrifugation, open the lid and air-dry for 2 - 5 min to completely remove the residual ethanol.
7. Transfer the adsorption column to a new 1.5 ml centrifuge tube (Self-prepared). Add 50 - 200 µl Elution Buffer to the center of the adsorption column membrane and incubate at room temperature for 2 - 5 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min.
   Note: The following steps can help to increase DNA yield.
   ▲ Pre-heat the Elution Buffer to 55℃ before elution.
   ▲ The solution from the first elution can be re-added to the adsorption column when carrying out the elution.
8. Discard the adsorption column and store the DNA products at -20℃. For long-term storage, please store at -70℃ to prevent degradation.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogged adsorption column	1.Excessive amount of sample	Please input samples according to the compatible range.
	2.Decrease in Proteinase K activity	Replace with new Proteinase K, and Proteinase K is recommended to store at 2 ~ 8℃.
	3.Insufficient sample lysis	Increase the incubation time in a 56℃ water bath and mix by inverting more frequently.
Low DNA yield	1.Samples were frozen and thawed more than once	Avoid repeated freezing and thawing of samples. It is recommended to use fresh samples or samples that have been thawed only once.
	2.Insufficient lysis of animal tissue	The tissue should be chopped into pieces or ground with liquid nitrogen; Mix the sample thoroughly with Buffer ACL and Proteinase K; Appropriately increase the incubation time in the 56℃ water bath.
	3.Incomplete breakdown of Gram-positive bacteria cell walls	The amount of Lysozyme can be increased appropriately or the digestion time can be increased; Carry out the experiment according to complex bacteria operation procedure.
	4.Decrease in Proteinase K activity	Replace with new Proteinase K, and Proteinase K is recommended to store at 2 ~ 8℃.
	5.Lysis mixture is not fully transferred to the adsorption column	After addition of absolute ethanol, flocculent precipitate will appear in the solution, which must be transferred to the adsorption column together with the solution.
	6.Eluent issues	Please use the Elution Buffer provided in the kit. If ddH_2O or other eluents are used, ensure that the pH of the eluent is between 8.0 and 9.0. The Elution Buffer can be pre-heated to 55℃ before elution to help improve the DNA yield.
	7.Low elution efficiency	The eluent must be added to the center of the membrane; increase the elution volume or the number of elution times.
	8.Absolute ethanol was not added to Buffer WA/WB	Add the specified volume of absolute ethanol to Buffer WA and Buffer WB as indicated in the label.
Low DNA purity	1.Protein contamination	Buffer WA was not used for rinsing, or the correct volume of absolute ethanol was not added to Buffer WA. Add the specified volume of absolute ethanol as indicated in the label.
	2.Contamination with ions	Buffer WB was not used for rinsing, or rinsing was only carried out once. Please rinse twice with Buffer WB according to the instructions.
	3.RNA residue	RNase A was not added in the specified step, or the RNase A incubation time was too short. Please add RNase A in the step specified in the instructions. For samples with high RNA content, the incubation time can be increased appropriately.
	4.Ethanol residue	Before elution, the empty column was not centrifuged. Please open the lid and air-dry for 2 - 5 min to completely remove the ethanol after centrifugation.

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
Follow Us