# FastPure Microbiome DNA Isolation Kit

## DC502

Vazyme

Instructions for Use

Version 23.1

www.vazyme.com

# Contents

## 01/Product Description

This kit is intended for fast isolation and purification of host and microbiome DNA from blood, other biological fluids (alveolar lavage fluid, sputum, cerebrospinal fluid, etc.), swab, bacteria/fungi culture, and animal tissue. The kit combines chemical and mechanical lysis methods and can efficiently lyse bacterial and fungal cells with thick cell walls. The extraction process with this kit is safe, fast, and simple as neither toxic reagents (e.g., phenol and chloroform) nor time-consuming procedures — such as alcohol precipitation and RNase treatment—are required. While ensuring efficiency in nucleic acid extraction, the kit also minimizes the contamination of RNA, proteins, lipids, and other impurities. The isolated DNA is suitable for various downstream applications, including PCR, real-time PCR, metagenomic library preparation, and microarray analysis.

## 02/Components

| | Components | DC502-01 (50 rxns) |
|---|---|---|
| Box 1 | Lysis Buffer | 25 ml |
| | Binding Buffer | 10 ml |
| | Buffer WP | 25 ml |
| | Buffer WB | 10 ml |
| | Buffer EB | 3 × 1 ml |
| | PBS | 20 ml |
| | Lysis Tube | 50 |
| | Elution Tube 1.5 ml | 50 |
| | Collection Tube 2 ml | 50 |
| Box 2 | Proteinase K | 2 ml |
| Box 3 | FastPure DNA Column (each in a 2 ml Collection Tube) | 50 |

## 03/Storage

Store Box 1 at 15 ~ 25°C and transport at room temperature;

Store Box 2 at -30 ~ -15°C and transport at ≤ 0°C;

Store Box 3 at 2 ~ 8°C and adjust the shipping method according to the destination.

## 04/Applications

Blood samples: fresh or frozen anticoagulated whole blood (≤ 400 µl);

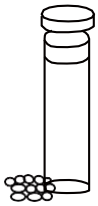
Biological fluid samples: fresh or frozen sputum, alveolar lavage fluid, cerebrospinal fluid, synovial fluid, pleural and peritoneal effusion, and vitreous humor (≤ $1 \times 10^7$ cells);

Swab samples: Nasal, pharyngeal, oral swabs, etc.;

Bacterial solutions: bacteria (≤ $2 \times 10^9$ cells) and fungi such as yeast (≤ $1 \times 10^9$ cells);

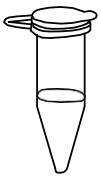
Tissue samples: ≤ 25 mg of animal tissue, such as heart, liver, or spleen, prepared by liquid nitrogen cryogenic grinding.

## 05/Mechanism & Workflow



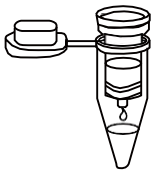
**Sample pretreatment**

- Sample volume is ≤ 400 µl (blood, other biological fluids, or swab):
  Make up the volume to 400 µl with PBS, add 100 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer, and disrupt the cells with glass beads for 10 min.
- Sample volume is > 400 µl (biological fluids, swab, or bacteria/fungal samples):
  Centrifuge at 10,200 rpm (10,000 × g) for 3 min to collect the sample, add 500 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer, and disrupt the cells with glass beads for 10 min.
- Tissue samples:
  Add 500 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer, and disrupt the cells with glass beads for 10 min.



**Adjust the binding environment:** Add 300 µl of ethanol absolute and vortex to mix well.

**Adsorption of genomic DNA:** Transfer the mixture to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min;
**Removal of proteins, RNA, etc.:** Add 500 µl of Buffer WP and centrifuge at 10,200 rpm (10,000 × g) for 1 min;
**Removal of salt ions:** Add 600 µl of Buffer WB and centrifuge at 13,300 rpm (17,000 × g) for 3 min;
**Removal of ethanol:** Centrifuge empty column at 13,300 rpm (17,000 × g) for 1 min.



**Elution of genomic DNA:** Add 50 µl of Buffer EB, leave at room temperature for 2 - 5 min, and centrifuge at 13,300 rpm (17,000 × g) for 1 min.

## 06/Self-prepared Materials

Absolute Ethanol
Nuclease-free pipette tips
Vortex mixer
Thermostatic water bath
High-speed centrifuge
Homogenizer (optional): FastPrep24 (MP Biomedicals, Cat. No. 116004500) or equivalent.

## 07/Notes

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

1. When using the kit, wear a lab coat, disposable latex gloves, and a disposable mask.
2. Pathogen samples should be processed in a biosafety cabinet.
3. Use Nuclease-free pipette tips and Nuclease-free centrifuge tubes to avoid contamination by exogenous microorganisms and nucleic acids.
4. Before your first use, add 40 ml of ethanol absolute to Buffer WB as indicated on the label of the reagent bottle, and mark accordingly.
5. Check for precipitates in Lysis Buffer and Binding Buffer before use. Redissolve any precipitate in a 37°C water bath. Mix well before use.

## 08/Experiment Process

■ Before your first use, add 40 ml of ethanol absolute to Buffer WB as indicated on the label of the reagent bottle, and mark accordingly.

■ Check for precipitates in Lysis Buffer and Binding Buffer before use. Redissolve any precipitate in a 37°C water bath. Mix well before use.

### 08-1/Sample Volume ≤ 400 µl (blood, other biological fluids, or swab)

1. Take 400 µl of blood, other biological fluids, or swab into a Lysis Tube, add 100 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer, and vortex to mix well.
   ▲ Make up the volume to 400 µl with PBS if the sample volume is < 400 µl.
2. Lyse the cells using one of the following recommended methods:
   a. Vortex the Lysis Tube on a vortex mixer at maximum speed for 10 min.
   b. Place the Lysis Tube in a homogenizer and lyse cells by running an appropriate program (e.g., use FastPrep-24 from MP Biomedicals, 6.5 m/sec, on 90 sec, off 3 min, 2 cycles).
   ▲ Floccules that do not affect the extraction efficiency may appear in this step.
   ▲ When using a homogenizer from other suppliers, select a recommended program.
3. Heat the Lysis Tube in a 70°C water bath for 5 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min to remove the foam, then transfer all the supernatant into a new 1.5 ml Nuclease-free centrifuge tube.
   ▲ Do not pipette glass beads when transferring the supernatant.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, increase the centrifugation time to remove the foam.
4. Add 300 µl of absolute ethanol to the centrifuge tube of Step 3, shake to mix well, and collect the liquid on the tube cap by brief centrifugation.
   ▲ Following the addition of absolute ethanol, the solution may become turbid or flocculent as a normal reaction. Shake to mix the solution well and directly proceed to the next step.
5. Transfer 600 µl of the mixture (including floccules) of Step 4 to a FastPure DNA Column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the flow-through. Repeat this step to run all mixture (including floccules) obtained in Step 4 through the FastPure DNA Column.
6. Discard the flow-through and place the FastPure DNA Column in a new 2 ml Collection Tube. Add 500 µl of Buffer WP along the tube wall to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
7. Discard the flow-through and place the FastPure DNA Column in the Collection Tube. Add 600 µl of Buffer WB (check whether ethanol absolute has been added before use) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, and discard the flow-through.
8. Place the spin column in the Collection Tube and centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
9. Transfer the spin column into a new 1.5 ml Elution Tube, and add 50 µl of Buffer EB to the center of the spin column without touching the column. Leave the column at room temperature for 2 - 5 min and centrifuge at 13,300 rpm (17,000 × g) for 1 min.
   ▲ You may pre-heat Buffer EB to 55°C to increase the elution efficiency.
   ▲ You may load the eluate from the first elution back onto the spin column for a second elution to increase the eluate concentration.
10. Discard the spin column. The eluate can be used directly for downstream experiments or stored at -20°C.
    ▲ The VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme #ND617) is recommended for downstream DNA library preparation.

### 08-2/Sample Volume > 400 µl (biological fluids, swab, or bacteria/fungal samples)

1. Take biological fluids, swab, or bacteria/fungal samples into a Lysis Tube, centrifuge at 10,200 rpm (10,000 × g) for 3 min, and remove as much of the culture supernatant as possible with a pipette. If the sample needs to be collected multiple times, repeat this step every time.
   ▲ The quantity of bacteria can be measured with a spectrophotometer.
   ▲ Do not pipette glass beads when removing the supernatant with a pipette.
2. Add 500 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer to the Lysis Tube, vortex to mix well, and lyse the cells using one of the following recommended methods:
   a. Vortex the Lysis Tube on a vortex mixer at maximum speed for 10 min.
   b. Place the Lysis Tube in a homogenizer and lyse cells by running an appropriate program (e.g., use FastPrep-24 from MP Biomedicals, 6.5 m/sec, on 90 sec, off 3 min, 2 cycles).
   ▲ For difficult-to-break microorganisms, extend the cell disruption time appropriately.
   ▲ When using a homogenizer from other suppliers, select a recommended program.
   ▲ Floccules that do not affect the extraction efficiency may appear in this step.
3. Heat the Lysis Tube in a 70°C water bath for 5 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min to remove the foam, then transfer all the supernatant into a new 1.5 ml Nuclease-free centrifuge tube.
   ▲ Floccules that appear in this step do not affect the extraction efficiency. Mix well by gently pipetting up and down, and transfer the floccules with the supernatant without pipetting the glass beads.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, increase the centrifugation time to remove the foam.
4. Add 300 µl of absolute ethanol to the centrifuge tube of Step 3, shake to mix well, and collect the liquid on the tube cap by brief centrifugation.

▲ Following the addition of absolute ethanol, the solution may become turbid or flocculent as a normal reaction. Shake to mix the solution well and directly proceed to the next step.

5. Transfer 600 µl of the mixture (including floccules) of Step 4 to a FastPure DNA Column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the flow-through. Repeat this step to run all mixture (including floccules) obtained in Step 4 through the FastPure DNA Column.
6. Discard the flow-through and place the FastPure DNA Column in a new 2 ml Collection Tube. Add 500 µl of Buffer WP along the tube wall to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
7. Discard the flow-through and place the FastPure DNA Column in the Collection Tube. Add 600 µl of Buffer WB (check whether absolute ethanol has been added before use) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, and discard the flow-through.
8. Place the spin column in the Collection Tube and centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
9. Transfer the spin column into a new 1.5 ml Elution Tube, and add 50 µl of Buffer EB to the center of the spin column without touching the column. Leave the column at room temperature for 2 - 5 min and centrifuge at 13,300 rpm (17,000 × g) for 1 min.
   ▲ You may pre-heat Buffer EB to 55°C to increase the elution efficiency.
   ▲ You may load the eluate from the first elution back onto the spin column for a second elution to increase the eluate concentration.
10. Discard the spin column. The eluate can be used directly for downstream experiments or stored at -20°C.
    ▲ The VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme #ND617) is recommended for downstream DNA library preparation.

### 08-3 /Tissue Samples

1. Take ≤ 25 mg of animal tissue ground in liquid nitrogen to a Lysis Tube, add 500 µl of Lysis Buffer, 40 µl of Proteinase K, then 200 µl of Binding Buffer, vortex to mix well, and lyse the cells using one of the following recommended methods:
   a. Vortex the Lysis Tube on a vortex mixer at maximum speed for 10 min.
   b. Place the Lysis Tube in a homogenizer and lyse cells by running an appropriate program (e.g., use FastPrep-24 from MP Biomedicals, 6.5 m/sec, on 90 sec, off 3 min, 2 cycles).
   ▲ For difficult-to-break tissue samples, extend the cell disruption time appropriately.
   ▲ When using a homogenizer from other suppliers, select a recommended program.
   ▲ Floccules that do not affect the extraction efficiency may appear in this step.
2. Heat the Lysis Tube in a 70°C water bath for 5 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min to remove the foam, then transfer all the supernatant into a new 1.5 ml Nuclease-free centrifuge tube.
   ▲ Floccules that appear in this step do not affect the extraction efficiency. Mix well by gently pipetting up and down, and transfer the floccules with the supernatant without pipetting the glass beads.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, increase the centrifugation time to remove the foam.
3. Add 300 µl of absolute ethanol to the centrifuge tube of Step 2, shake to mix well, and collect the liquid on the tube cap by brief centrifugation.
   ▲ Following the addition of absolute ethanol, the solution may become turbid or flocculent as a normal reaction. Shake to mix the solution well and directly proceed to the next step.
4. Transfer 600 µl of the mixture (including floccules) of Step 3 to a FastPure DNA Column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, and discard the flow-through. Repeat this step to run all mixture (including floccules) obtained in Step 3 through the FastPure DNA Column.
5. Discard the flow-through and place the FastPure DNA Column in a new 2 ml Collection Tube. Add 500 µl of Buffer WP along the tube wall to the spin column and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
6. Discard the flow-through and place the FastPure DNA Column in the Collection Tube. Add 600 µl of Buffer WB (check whether absolute ethanol has been added before use) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, and discard the flow-through.
7. Place the spin column in the Collection Tube and centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
8. Transfer the spin column into a new 1.5 ml Elution Tube, and add 50 µl of Buffer EB to the center of the spin column without touching the column. Leave the column at room temperature for 2 - 5 min and centrifuge at 13,300 rpm (17,000 × g) for 1 min.
   ▲ You may pre-heat Buffer EB to 55°C to increase the elution efficiency.
   ▲ You may load the eluate from the first elution back onto the spin column for a second elution to increase the eluate concentration.
9. Discard the spin column. The eluate can be used directly for downstream experiments or stored at -20°C.
   ▲ The VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme #ND617) is recommended for downstream DNA library preparation.

## 09/FAQ & Troubleshooting

| Question | Cause | Solution |
|---|---|---|
| Sputum treatment method | Sputum samples are too viscous and need to be liquefied before extraction. | If the sample is white foamy thin sputum, take an appropriate volume of it, add five volumes of acetylcysteine solution (10 g/L), shake to mix well and liquefy for 30 min - 1 h, and proceed to the extraction steps. If the |

| | | |
|---|---|---|
| | | sample is white or yellow-white viscous sputum, yellow sputum with blood streaks, or bloody sputum, take an appropriate volume of it, add 10 volumes of acetylcysteine solution (10 g/L), shake to liquefy for 30 min - 1 h, and proceed to the extraction steps. |
| Low DNA Yield | Incomplete cell disruption | Extend the mechanical lysis time appropriately for complete cell disruption. |
| | Incomplete transfer of the lysate to the spin column | Following the addition of absolute ethanol after cell lysis, floccules may appear in the solution and should be transferred together to the spin column. |
| | Low elution efficiency | Pre-heat Buffer EB to 55°C before use and increase the volume and number of elutions. |
| | Buffer WB not supplemented with ethanol absolute | Add a specified volume of ethanol absolute to Buffer WB as indicated on the label of the reagent bottle. |
| | Protein contamination | Make sure to rinse with Buffer WP and Buffer WB. |
| Low DNA Purity | Ion contamination | The spin column may not have been rinsed with Buffer WB. Rinse it twice with Buffer WB. |
| | Ethanol residue | Before elution, centrifuge the empty column to remove ethanol. |

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