FastPure Host Removal and Microbiome DNA Isolation Kit

DC501

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

This kit is applicable for selective removal of host nucleic acid from biological fluid samples (blood, alveolar lavage fluid, sputum, cerebrospinal fluid, swabs and others) and for rapid isolation and purification of microbial DNA. The Buffer SL in the kit selectively lyses host cells, and the released host nucleic acid is rapidly degraded by Ultra Nuclease, while bacteria, fungi and other microorganisms have almost no loss in this process. This kit is also applicable for directly extractting DNA of all species from a variety of complex samples including bacteria, fungi, viruses and other microorganisms, as well as the hosts, without removal of host nucleic acid. The DNA isolated by this kit is applicable for a variety of downstream applications, including PCR, Real-Time PCR, metagenomic library preparation, chip analysis and others.

02/Components

	Components	DC501-01 (50 rxns)
BOX 1	Buffer SL	5 × 5 ml
	Ultra Nuclease	250 µl
	Proteinase K	3 ml
	Buffer NAD	9.5 ml
BOX 2	Lysis Buffer	25 ml
	Binding Buffer	10 ml
	Buffer WP	25 ml
	Buffer WB	10 ml
	Buffer EB	3 × 1 ml
	PBS	50 ml
	Lysis Tube	50
	Sample Tube 2 ml	50
	Elution Tube 1.5 ml	60
	Collection Tube 2 ml	50
BOX 3	FastPure DNA Column (each in a 2 ml Collection Tube)	50

03/Storage

Store BOX 1 at -30 ~ -15°C and transport at ≤0°C.

Store BOX 2 at 15 ~ 25°C and transport at room temperature.

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

04/Applications

Process Selection	Compatible Amount of Sample
08-1 Host removal + microbiome DNA extraction	Blood samples: fresh anticoagulant whole blood (≤1 ml) Biological fluid samples: fresh sputum, alveolar lavage fluid, cerebrospinal fluid, synovial fluid, hydrothorax and ascites, the eye-intraocular fluid and others (host cells ≤1 × 10^7) Swab samples: fresh swab collection liquid from the pharynx, nose, oral cavity and others (host cells ≤1 × 10^7)
08-2 Host non-removal + host and microbiome total DNA extraction	Blood samples: fresh anticoagulant whole blood (≤400 μl) Biological fluid samples: fresh sputum, alveolar lavage fluid, cerebrospinal fluid, synovial fluid, hydrothorax and ascites, the eye-intraocular fluid and others (host cells ≤1 × 10^7) Swab samples: fresh swab collection liquid from the pharynx, nose, oral cavity and others (host cells ≤1 × 10^7) Bacterial liquid samples: bacteria (≤2 × 10^9), yeast cells (≤1 × 10^9)

05/Self-prepared Materials

Absolute ethanol, Nuclease-free tip, 1.5 ml Nuclease-free centrifugation tube, vertical mixer, vortex oscillator, thermostatic water bath, high-speed centrifuge.

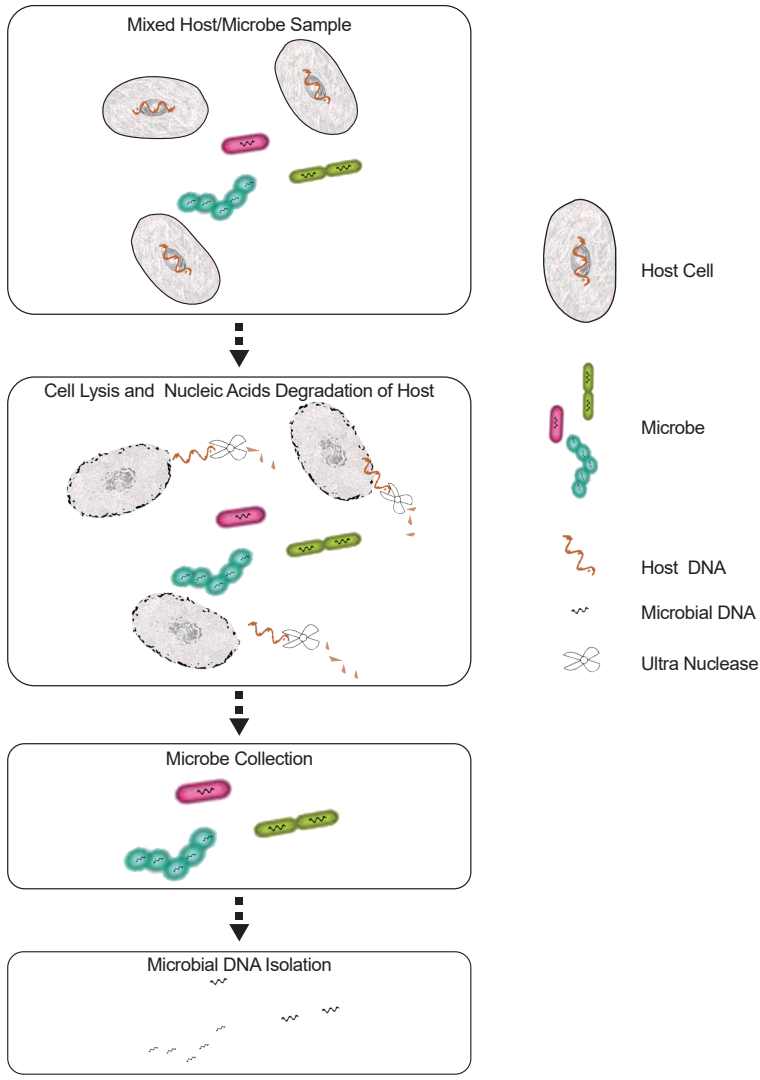
Homogenizer: such as FastPrep-24 (MP Company Cat. No. 116004500) or other equivalent instruments.

06/Notes

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

1. When using this kit, please take protective measures, wear a laboratory coat, disposable latex gloves and a mask.
2. When this kit is used for host nucleic acid removal, it is necessary to ensure that the samples are fresh and have not been frozen or thawed.
3. Pathogen sample processing should be carried out in a biological safety cabinet.
4. Please use Nuclease-free pipette tips and Nuclease-free centrifugation tubes to avoid contamination by exogenous microorganisms and exogenous nucleic acids.
5. Before first use, please refer to the bottle label to add 40 ml of absolute ethanol to Buffer WB, and label accordingly.
6. Please check if there is any precipitates in the Lysis Buffer and Binding Buffer before use. If precipitates have formed, dissolve them in 37°C water bath and mix thoroughly before use.
7. Before first test, if "08-1/Host Removal and Microbial DNA Extraction Standard Operating Procedures" is intended for host removal and microbial DNA extraction of the sample, it is recommended to set up a parallel control group and extract sample total DNA using "08-2/Host Non-removal and Microbial and Host Total DNA Extraction Standard Operating Procedures" to evaluate host removal rate.

07/Mechanism & Workflow



Mechanism of FastPure Host Removal and Microbiome DNA Isolation Kit

1 ml of fresh sample (blood, sputum, alveolar lavage fluid, swabs and others)

Part I Removal of Host nucleic acid

Step I Lyse host cells

Add 500 µl of Buffer SL, invert it at room temperature for 20 min

Step II Degrade host nucleic acid

Add 190 µl of Buffer NAD and 5 µl of Ultra Nuclease and place in water bath at 37°C for 10 min, then add 20 µl of Proteinase K and place in water bath at 56°C for 10 min

Part II Isolation of Microbiome DNA

Step III Lyse microbiome cells

Add Lysis Buffer (Select the addition volume according to different processes)
Add 40 µl of Proteinase K
Add 200 µl of Binding Buffer, mechanical crushing with glass beads

Step IV Adjust DNA binding environment

Add absolute ethanol (select the addition volume according to different processes), fully mix by vortex-mixing

Step V Purify DNA with column

Centrifuge at 10,200 rpm (10,000 × g) for 1 min

Step VI Remove residual protein

Add 500 µl Buffer WP
Centrifuge at 10,200 rpm (10,000 × g) for 1 min

Step VII Remove residual salt/ethano

Add 600 µl Buffer WB
Centrifuge at 13,300 rpm (17,000 × g) for 3 min
Centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min

Step VIII Elute DNA

Add 50 µl Buffer EB, place at room temperature for 2 min to 5 min, centrifuge at 13,300 rpm (17,000 × g) for 1 min

Microbiome DNA without host nucleic acid

Experiment Process Outline of FastPure Host Removal and Microbiome DNA Isolation Kit

08/Experiment Process

- Please perform this step if host nucleic acid removal is required for the samples: 08-1/Host Removal and Microbial DNA Extraction Standard Operating Procedures. If DNA of all species is extracted directly without host nucleic acid removal, refer to 08-2/Host Non-removal and Microbial and Host Total DNA Extraction Standard Operating Procedures.
- Before first use, please refer to the bottle label and add 40 ml of absolute ethanol to Buffer WB, and label accordingly.
- Please check if there is any precipitates in the Lysis Buffer and Binding Buffer before use. If precipitates have formed, dissolve them in 37°C water bath and mix thoroughly before use.

08-1/Host Removal and Microbial DNA Extraction Standard Operating Procedures

08-1 Part I Host Removal

1. Add 1 ml of sample (fresh and not frozen and thawed) into the Sample Tube, invert and mix thoroughly with 500 µl Buffer SL at room temperature for 20 min.
   ▲ If the sample is <1 ml, supplement to 1 ml with PBS. If the sample is >1 ml, collect samples by centrifugation at 10,200 rpm (10,000 × g) for 1 min, then resuspend the sample using 1 ml of PBS.
   ▲ If frozen and thawed samples are used, microbial DNA may be lost due to the rupture of microbial cells.
2. Centrifuge the Sample Tube at 10,200 rpm (10,000 × g) for 3 min, and carefully discard the supernatant using a pipette.
   ▲ Do not touch the precipitates during pipetting to avoid loss of microbial DNA.
   ▲ Remove the supernatant as much as possible to avoid host nucleic acid residue.
3. Add 190 µl of Buffer NAD and 5 µl of Ultra Nuclease to the Sample Tube, then mix by vortex-mixing, and place it in water bath at 37°C for 10 min.
4. Add 20 µl Proteinase K to the Sample Tube, then mix by vortex-mixing, and place in a water bath at 56°C for 10 min. After the reaction is completed, **brief centrifugation** is performed, followed by **08-1 Part II Microbial DNA Extraction immediately.**
   ▲ At this step, host nucleic acid removal has been completed, please proceed to the subsequent microbial DNA extraction immediately to avoid affecting the extraction yield.

08-1 Part II Microbial DNA Extraction

1. Transfer all the mixture in **08-1 Part I** step 4 to the Lysis Tube.
   ▲Please transfer all the mixture to aviod product loss.
2. Add 200 µl of Lysis Buffer, 40 µl of Proteinase K, and 200 µl of Binding Buffer to the Lysis Tube in order, mix by vortex-mixing. Perform cell lysis using one of the two following methods:

a. Place the Lysis Tube on the vortexer and vortex at the maximum speed (≥3,000 rpm) for 10 min.

b. Place the Lysis Tube on the homogenizer and select a suitable procedure for lysis, such as MP FastPrep-24, 4°C 6.5 m/sec, on 90 sec, off 3 min, 2 cycles.
   ▲ The floccules that may appear in this step will not affect the extraction efficiency.
   ▲ If other brands of homogenizer are used, please select the recommended procedure of the instrument.

3. Place Lysis Tube at 70°C water bath for 5 min.
4. Take out the Lysis Tube, centrifuge at 10,200 rpm (10,000 × g) for 1 min to eliminate foam, then transfer 500 µl of supernatant to a new 1.5 ml centrifugation tube.
   ▲ When transferring the supernatant, do not absorb the glass beads.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, please prolong the centrifugation time to eliminate foam.

5. Add 200 μl of absolute ethanol to the centrifugation tube in step 4, fully mix by vortex-mixing, centrifuge briefly to collect the liquid on the inner tube wall lid.
   ▲ IIt is normal for the solution to turn turbid or to contain floccules after adding absolute ethanol. Mix the solution well by vortex-mixing and use it directly for the next step.
6. Transfer **all the mixture (including floccules) in step 5** to the FastPure DNA Column absorption column, centrifuge at 10,200 rpm (10,000 × g) for 1 min.
7. Discard the filtrate, place the FastPure DNA Column absorption column into a new 2 ml Collection Tube. Add 500 μl of Buffer WP into the absorption column along the tube wall, centrifuge at 10,200 rpm (10,000 × g) for 1 min.
8. Discard the filtrate and place the FastPure DNA Column in the collection tube. Add 600 μl of Buffer WB (**please ensure that absolute ethanol has been added**) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, then discard the filtrate.
9. Place the absorption column in the collection tube, centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
10. Transfer the absorption column to a new 1.5 ml Elution Tube, add 50 μl of Buffer EB to the center of the adsorption column. Place at room temperature for 2 min to 5 min, centrifuge at 13,300 rpm (17,000 × g) for 1 min.
    ▲ Pre-heat Buffer EB to 55°C to improve the elution efficiency.
    ▲ The solution from the first elution can be added to the adsorption column for the secondary elution to increase the concentration of eluted products.
11. Discard the adsorption column, and directly apply the eluted product in downstream experiments or store at -20°C.
    ▲ UltraClean Universal Plus DNA Library Prep Kit for Illumina V3 (Vazyme #UND637) is recommended for downstream DNA library preparation.
    ▲ If the proportion of host cells in the sample is high, it is a normal phenomenon that the concentration of the extracted product is lower than the lower limit of Qubit detection. The maximum volume compatible with the library preparation kit can be used for experiments.
    ▲ For the first test, “08-1/Host Removal and Microbial DNA Extraction Standard Operating Procedures” is intended for host removal and microbial DNA extraction of the sample, it is recommended to set up a parallel control group and extract sample total DNA using “08-2/Host Non-removal and Microbial and Host Total DNA Extraction Standard Operating Procedures” to evaluate host removal rate.

08-2/Host Non-removal and Microbial and Host Total DNA Extraction Standard Operating Procedures

◇ Blood samples (≤400 μl)

1. Add 400 μl of blood samples to Lysis Tube, then add 100 μl of Lysis Buffer, 40 μl of Proteinase K, and 200 μl of Binding Buffer in order, and fully mix by vortex-mixing.
   ▲ If the solution is <400 μl, supplement to 400 μl with PBS.
2. Perform cell lysis using one of the two following methods:

a. Place the Lysis Tube on the vortexer and vortex at the maximum speed for 10 min.

b. Place the Lysis Tube on homogenizer and select a suitable procedure for lysis, such as MP FastPrep-24, 4°C 6.5 m/sec, on 90 sec, off 3 min, 2 cycles.
   ▲ The floccules that may appear in this step will not affect the extraction efficiency.
   ▲ If other brands of homogenizers are used, please select the recommended procedure of the instrument.

3. Place the Lysis Tube in a water bath at 70°C for 5 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min to eliminate foam, then transfer all the supernatant to a new 1.5 ml centrifugation tube.
   ▲ When transferring the supernatant, do not absorb the glass beads.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, please prolong the centrifugation time to eliminate foam.
4. Add 300 µl of absolute ethanol to the centrifugation tube in step 3, fully mix by vortex-mixing, then centrifuge briefly to collect the liquid on the inner wall of the collection tube.
   ▲ It is normal for the solution to turn turbid or to contain floccules after adding absolute ethanol, and mix the solution thoroughly by vortex-mixing and use it directly for the next step.
5. Transfer 600 µl of the mixture (including floccules) in step 4 to the FastPure DNA Column absorption column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, then discard the filtrate. Repeat this step, transfer all the remaining mixture (including floccules) in step 4 to the FastPure DNA Column.
6. Discard the filtrate, place the FastPure DNA Column absorption column in a new 2 ml collection tube. Add 500 µl of Buffer WP into the absorption column along the tube wall, centrifuge at 10,200 rpm (10,000 × g) for 1 min.
7. Discard the filtrate and place the FastPure DNA Column in the collection tube. Add 600 µl of Buffer WB (please ensure that absolute ethanol has been added) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, then discard the filtrate.
8. Place the absorption column in the collection tube, centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
9. Transfer the absorption column to a new 1.5 ml Elution Tube, add 50 µl of Buffer EB to the center of the adsorption column. Place at room temperature for 2 min to 5 min, centrifuge at 13,300 rpm (17,000 × g) for 1 min.
   ▲ Pre-heat Buffer EB to 55°C to improve the elution efficiency.
   ▲ The solution from the first elution can be added to the adsorption column for the secondary elution to increase the concentration of eluted products.
10. Discard the adsorption column, and directly apply the eluted product in downstream experiments or store at -20°C.
    ▲ UltraClean Universal Plus DNA Library Prep Kit for Illumina V3 (Vazyme #UND637) is recommended for downstream DNA library preparation.

◇ Biological fluid, swab or microbial culture samples

1. Take and place the biological fluid, swab or microbial culture sample into the Lysis Tube, centrifuge at maximum speed for 5 min (>12,000 rpm (13,400 × g)), discard the culture medium supernatant as much as possible. If multiple sample collections are required, please repeat this step.
   ▲ The number of bacteria can be measured with a spectrophotometer.
   ▲ When discarding the supernatant with a pipette, do not absorb the glass beads.
   ▲ If the biological fluid sample is ≤400 µl, the extraction protocol of ◇blood sample (≤400 µl) can be chosen.
2. Add 500 µl of Lysis Buffer, 40 µl Proteinase K, and 200 µl Binding Buffer to the Lysis Tube in order, mix by vortex-mixing. Perform cell lysis using one of the two following methods:

a. Place the Lysis Tube on the vortexer and vortex at the maximum speed for 10 min.

b. Place the Lysis Tube on the homogenizer and select a suitable procedure for lysis, such as MP FastPrep-24, 4°C 6.5 m/sec, on 90 sec, off 3 min, 2 cycles.
   ▲ For microorganisms whose cell walls are difficult to disrupt, prolong the disruption time appropriately.
   ▲ If other brands of homogenizers are used, please select the recommended procedure of the instrument.
   ▲ The floccules that may appear in this step will not affect the extraction efficiency.
3. Place the Lysis Tube in water bath at 70°C for 5 min, centrifuge at 10,200 rpm (10,000 × g) for 1 min to eliminate foam, and transfer all the supernatant to a new 1.5 ml Nuclease-free centrifugation tube.
   ▲ If floccules appeared in step 3 will not affect the extraction efficiency, gently pipette up and down, and transfer the floccules together, and avoid absorbing the glass beads.
   ▲ If there is still foam after centrifugation at 10,200 rpm (10,000 × g) for 1 min, please prolong the centrifugation time to eliminate foam.
4. Add 300 µl of absolute ethanol to the centrifugation tube in step 3, fully mix by vortex-mixing, then centrifuge briefly to collect the liquid on the inner wall of the collection tube.
   ▲ It is normal for the solution to turn turbid or to contain floccules after adding absolute ethanol and mix the solution thoroughly by vortex-mixing and use it directly for the next step.
5. Transfer 600 µl of the mixture (including floccules) in step 4 to the FastPure DNA Column absorption column, centrifuge at 10,200 rpm (10,000 × g) for 1 min, then discard the filtrate. Repeat this step, transfer all the remaining mixture (including floccules) in step 4 to FastPure DNA Column.
6. Discard the filtrate, place the FastPure DNA Column absorption column in a new 2 ml collection tube. Add 500 µl of Buffer WP along the tube wall to the absorption column, and centrifuge at 10,200 rpm (10,000 × g) for 1 min.
7. Discard the filtrate and place the FastPure DNA Column in the collection tube. Add 600 µl of Buffer WB (please ensure that absolute ethanol has been added) along the tube wall, centrifuge at 13,300 rpm (17,000 × g) for 3 min, then discard the filtrate.
8. Place the absorption column in the collection tube, centrifuge the empty column at 13,300 rpm (17,000 × g) for 1 min.
9. Transfer the absorption column to a new 1.5 ml Elution Tube, add 50 µl of Buffer EB to the center of the adsorption column. Place at room temperature for 2 min to 5 min, centrifuge at 13,300 rpm (17,000 × g) for 1 min.
   ▲ Pre-heat Buffer EB to 55°C to improve the elution efficiency.
   ▲ The solution from the first elution can be added to the adsorption column for the secondary elution to increase the concentration of eluted products.
10. Discard the adsorption column, and directly apply the eluted product in downstream experiments or store at -20°C.
   ▲ UltraClean Universal Plus DNA Library Prep Kit for Illumina V3 (Vazyme #UND637) is recommended for downstream DNA library preparation.

09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Low host nucleic acid removal rate	Excessive sample input volume	Reduce sample input volume
	Insufficient sample lysis	To prolong the time of inverting mix at room temperature in 08-1 Part I step 1
	Insufficient degradation of host nucleic acid	To increase the ultra nuclease volume used or prolong the water bath time in 08-1 Part I step 3
Non-specific loss of microorganisms	The supernatant discarding step causes the loss of microorganisms	In 08-1 Part I step 2, when the supernatant is discarded, please do not touch the precipitates to avoid loss of microorganisms
	In 08-1 Part I step 4, Proteinase K digestion time is too long	Please operate according to the time specified in the manual
	Repeated freezing and thawing of sample	Please use fresh samples that have not been frozen and thawed
Low DNA yield	Insufficient disruption of the cell wall	Appropriately prolong the time of mechanical lysis to ensure complete disruption of the cell wall
	Lysis mixture not fully transferred to the adsorption column	After cell lysis and addition of the absolute ethanol, floccules will appear in the solution, please transfer the floccules to the adsorption column together with the solution
	Low elution efficiency	Pre-heat Buffer EB to 55°C before use, to increase the elution volume and times of elution
	No absolute ethanol added to Buffer WB	Add the specified volume of absolute ethanol to Buffer WB as indicated on the bottle labeling
Low DNA purity	Protein contamination	Buffer WP and Buffer WB is not used for rinsing
	Ions contamination	Buffer WB is not used for rinsing, and rinsing with Buffer WB twice is recommended
	Ethanol residue	Before the elution with the adsorption column, the empty column is not centrifuged to remove the ethanol

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