EasyPure® Microbiome DNA Isolation Kit

Cat. No. EE401
Version No. Version 1.0

Description

The kit is designed for the isolation and purification of host and microbial genomic DNA from biological samples such as blood, alveolar lavage fluid, liquefied sputum, nasopharyngeal or oropharyngeal swabs, pleural ascites, cerebrospinal fluid, amniotic fluid, etc. Microbial DNA is specifically bound to silica-based column, and the purified DNA is fully compatible with a variety of downstream applications, including PCR, qPCR, metagenomic or 16S rDNA library construction.
The kit can be used with *Trans*NGS® Host DNA Depletion Kit (EH301) to remove host nucleic acids.

Features

• High quality, high yield, meet a variety of downstream tests
• Low background, reduce false positives

Kit Contents

	Component	EE401-01 (50 rxns)
BOX 1	EasyPure DNA Columns with Collection Tubes	50
BOX 2	Proteinase K (20 mg/ml)	700 μl×3
	Lysis Buffer 47（LB 47）	23 ml
	Binding Buffer 47（BB 47）	12 ml
	Clean Buffer 47（CB 47）	15 ml
	Wash Buffer 47（WB 47）	8 ml
	Elution Buffer（EB）	6 ml
	1×PBS	10 ml
	Lysis Tube	50
	Elution Tube（1.5 ml）	50

Storage Conditions

EasyPure® Microbiome DNA Isolation Kit	Storage Conditions
BOX1	at 4°C for one year
BOX2	at 15-25°C for one year

Customer-Supplied Reagent and Equipments

Absolute ethanol (analytically pure)
Nuclease-free pipette tips with filter element
Rotator (e.g. DLAB®MX-RD-E, etc.)
Vortex mixer (e.g. Kylin Bell® Vortex-10, etc.)
Sample homogenizer (e.g. MP® FastPrep-96™, etc.)
High-speed centrifuge (speed≥ 13,000 rpm)

Sample Requirements

• Make the sample is fresh before use, as repeated freeze-thaw will disrupt microbial integrity, resulting in loss of exposed microbial DNA when using host nucleic acid removal products.
• Be sure to use sterile media when collecting and storing samples, and open the sample storage media in a clean area to avoid contamination during sample handling.

Sample Type	Recommended Dosage
Blood sample (anticoagulant whole blood)	≤400 μl
Biological fluid samples (alveolar lavage fluid, cerebrospinal fluid, pleural ascites, serum, plasma, liquefied sputum, etc.)	Host cells ≤ 1×10^7
Swabs (nasal, pharynx and mouth)	Host cells ≤ 1×10^7
Bacterial samples	Number of bacteria ≤ 1×10^9

Procedures for Microbial and Host Total DNA Extraction

Please prepare the following before using the kit

- If precipitate appears in Lysis Buffer 47 (LB47) and Binding Buffer 47 (BB47), please dissolve at 56oC before use.
- For first use, refer to the bottle labels, add 15 ml and 32 ml of absolute ethanol to Clean Buffer 47 (CB47) and Wash Buffer 47 (WB47) respectively, and label.

DNA Extraction Procedures

Part A. Sample handling

If host nucleic acid removal is performed with *Trans*NGS® Host DNA Depletion Kit (EH301), directly transfer the treated liquid to the Lysis Tube and operate according to the blood treatment protocol.

- **Blood**

1. Add 400 μl of sample to the Lysis Tube containing glass beads. Add 200 μl LB47, 40 μl Proteinase K, and 200 μl BB47 to the Lysis Tube sequentially. Mix by vortexing.
   *If the sample volume is not enough, use PBS to make up to 400 μl.
2. Recommend one of the following two methods for cell lysis (choose one of the two):
   (1) Place the Lysis Tube on the vortex mixer and vortex at the maximum speed for 10 min.
   (2) Place the Lysis Tube in the sample homogenizer to select the appropriate program (e.g. MP® FastPrep-96™ parameter settings can refer to 6.5 m/sec, 60 sec, off 5 min, 2 cycles).
   *The flocculent that may appear after the addition of reagents at this step do not affect the extraction efficiency.

- **Biological fluids, swabs, or microbial culture solutions**

1. Add samples to Lysis Tube containing glass beads, centrifuge at 12,000 rpm for 5 min, and remove the supernatant as much as possible. Depending on the experimental needs, this step can be repeated for multiple sample enrichments.
   *If the sample volume ≤ 400 μl, follow the blood sample processing protocol.
   *When discarding the supernatant with a pipette, do not aspirate the glass beads.
2. Add 400 μl LB47, 40 μl Proteinase K, and 200 μl BB47 to the Lysis Tube sequentially. Mix by vortexing.
   Recommend one of the following two methods for cell lysis (choose one of the two):
   (1) Place the Lysis Tube on the vortex mixer and vortex at the maximum speed for 10 min.
   (2) Place the Lysis Tube in the sample homogenizer to select the appropriate program (e.g. MP® FastPrep-96™ parameter settings can refer to 6.5 m/sec, 60 sec, off 5 min, 2 cycles).
   *The flocculent that may appear after the addition of reagents at this step do not affect the extraction efficiency.

Part B. DNA extraction

1. Incubate the Lysis Tube at 70°C for 5 min, centrifuge at 12,000 rpm for 1 min to eliminate foam, pipet the entire supernatant and transfer it to a new 1.5 ml centrifuge tube.
   *If flocculent appears in this step, use a pipette to gently pipet and mix well, transfer the flocculent together, do not aspirate the glass beads.
   *If there is still foam after 1 min of centrifugation, the centrifugation time can be increased.
2. Add 300 μl of absolute ethanol to the centrifuge tube, mix well by vortexing, and centrifuge briefly to collect the liquid on the inner wall of the tube lid.
   *If the addition of absolute ethanol appears turbid or flocculent, it is normal.

3. Transfer all the mixture (including flocculents) from step 2 to EasyPure DNA Columns and centrifuge at 12,000 rpm for 1 min.
4. Discard the flowthrough, add 500 μl of CB47 along the tube wall (confirm that absolute ethanol has been added before use) to the spin column, and centrifuge at 12,000 rpm for 1 min.
5. Discard the flowthrough, add 600 μl of WB47 along the tube wall (confirm that absolute ethanol has been added before use) to the spin column, centrifuge at 13,000 rpm for 3 min, and discard the flowthrough.
6. Reassemble the spin column with its collection tube, and centrifuge the empty column at 13,000 rpm for 1 min.
7. Transfer the spin column to a new 1.5 ml Elution Tube. Add 50-100 μl of EB dropwise to the center of the spin column. Incubate at room temperature for 2-5 minutes, and centrifuge at 13,000 rpm for 1 min.
   * Preheat EB to 55°C for better elution.
   *Adding the solution obtained from the first elution back into the spin column for the second elution can increase the elution yield.
8. Discard the spin column. The eluted product can be used directly for downstream experiments or stored at -20°C.

Common Problems and Solutions

Problem	Possible Cause	Solution
Microbial DNA loss	Microbial cell loss	Preferably use fresh samples and avoid freezing and thawing as much as possible
	Caused by discarding the supernatant	When removing the supernatant for biological fluids, swabs, or microbial culture solutions, do not touch the bottom precipitation
	Insufficient disruption of microbial cell wall	Follow the instructions strictly. Appropriately extend the time of mechanical wall breaking
	Not add absolute ethanol or add low concentration ethanol to WB	Add the specified volume of absolute ethanol correctly according to the bottle label
	EasyPure DNA Column was not incubated at room temperature for 2-5 minutes after EB was added	Follow the instructions strictly
Low extraction efficiency	Insufficient wall breaking or too many cells in the enriched sample	Appropriately extend the time of mechanical wall breaking or reduce the amount of sample input
Low DNA purity	Contaminated by impurity ions	Wash twice with WB
	Ethanol residue	Make sure that the EasyPure DNA Column does not touch the flowthrough before elution, or extend the centrifugation time of the empty column
Precipitation appears during the extraction process	Precipitate may appear in LB and BB after storage at low temperature or for a long time	If precipitation appears in LB or BB, it must be incubated at 56°C until the precipitation is completely dissolved before use
	After adding BB, the liquid in the tube has precipitation	After adding BB, there will only be a few cases of white flocculent precipitates, which will be dissolved under the subsequent incubation condition at 70°C

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