MagicPure® Microbiome DNA Isolation Kit

Please read the manual carefully before use

Catalog No. EC107-32

Version No. Version 1.0

Storage: at room temperature for one year (15-25°C), avoid frozen.

Description

MagicPure® 32 Microbiome DNA Isolation Kit is a reagent kit designed for efficient purification of host and microbial genomic DNA from various biological samples such as blood, bronchoalveolar lavage fluid, liquefied sputum, nasal or oropharyngeal swabs, pleural or peritoneal fluid, cerebrospinal fluid, and amniotic fluid. This kit utilizes silica-based magnetic beads for specific adsorption and enrichment of microbial DNA, yielding DNA suitable for various downstream applications including PCR, qPCR, metagenomic or 16S rDNA library construction experiments. The kit is compatible with 32 channel magnetic rod-based high-throughput automatic nucleic acid extraction instrument and can be used in conjunction with the *Trans*NGS® Host DNA Depletion Kit (EH301) to achieve host nucleic acid depletion.

Feature

• High-throughput extraction with simple operation and fast speed.

• High-quality, higher yield to meet various downstream analysis needs.

• Lower reagent background noise to reduce the risk of false positives.

Kit content

Component	EC107-32-11 (32 rxns)
Microbiome DNA Reagents	2 plates
Lysis Buffer 45（LB45）	7 ml
Proteinase K (20 mg/ml)	1.5 ml
Lysis Tube	32 tubes
8-Tip Comb	4 pieces

Microbiome DNA Reagents Plate content:

Column	Name	Volume
Column 1/7	Binding Buffer 45 for Plate（BB45 for Plate）	500 µl/well×16
Column 2/8	Magnetic Microbiome Beads for Plate	300 µl/well×16
Column 3/9	Clean Buffer 45 for Plate（CB45 for Plate）	700 µl/well×16
Column 4/10	Wash Buffer 45 for Plate（WB45 for Plate）	700 µl/well×16
Column 5/11	Wash Buffer 45 for Plate（WB45 for Plate）	700 µl/well×16
Column 6/12	Elution Buffer	100 µl/well×16

Self prepared reagent and equipment

Sterile 1×PBS (pH7.4) solution

Nuclease-free Pipette Tips with Filter

Vortex Mixer (e.g., KylinBell® Vortex-10 or similar)

High-Speed Centrifuge (speed ≥ 10,000 rpm)

Thermostatic Water Bath

Sample Requirements

• Ensure samples are fresh before use, avoiding repeated freeze-thaw cycles.

• Collect and store samples in sterile media, and open them in a clean area to prevent contamination during sample handling.

Sample type	Recommended volume
Blood sample (Anticoagulated whole blood)	≤200 μl
Biological fluid samples (such as bronchoalveolar lavage fluid, cerebrospinal fluid, pleural or peritoneal fluid, serum, plasma, liquefied sputum, etc.)	Host cell ≤1×10^7
Swab specimens (nasal, throat, oral swabs)	Host cell≤1×10^7
Bacterial liquid samples	Bacterial cell ≤1×10^9

Automated Extraction Procedure for Microbial and Host Total DNA

Preparation before using this kit:

- If there is precipitation in the Lysis Buffer 45 (LB 45), dissolve and mix thoroughly at 56°C before use.
- Take out the pre-packaged 96-well deep well plate (Microbiome DNA Reagents) from the kit, discard the outer packaging of the deep well plate, invert the 96-well deep well plate several times to resuspend the magnetic beads evenly, gently flick the deep well plate to concentrate the reagents and magnetic beads at the bottom of the wells (or use a deep well plate centrifuge, centrifuge at 500 rpm for no more than 1 minute).

Microbial DNA Extraction:

1. Sample Processing:

(1) Add 200 μl of the sample to a Lysis Tube, then add 200 μl of LB45 and 40 μl of Proteinase K sequentially. Place it on a vortex mixer and vortex at maximum speed for 10 minutes.

* If blood sample volume is insufficient, add PBS to make up to 200 μl.
* For biological fluids, swabs, microbial culture samples, etc., if the sample volume ≤ 200 μl, add PBS to make up to 200 μl. If 200 μl < sample volume ≤ 1 ml, centrifuge at 12000 rpm for 5 minutes, discard part of the supernatant, and use the remaining 200 μl for extraction.
* If conducting host depletion with *Trans*NGS® Host DNA Depletion Kit (EH301), transfer the processed liquid directly to the Lysis Tube and proceed as per this manual.

(2) Place the Lysis Tube in a 70°C water bath for 5 minutes, then centrifuge at 12000 rpm for 1 minute to eliminate foam.

* If foam persists after 1 minute of centrifugation, increase the centrifugation time to eliminate it.

2. Transfer all the supernatant into 96 deep-well plate wells of column 1/7.

* When transferring the supernatant, do not pipette out the glass beads.

3. Insert the tip combs into the slot of the 32-channel automated nucleic acid extraction instrument.

4. Run the automated microbial DNA extraction program on the 32-channel automated nucleic acid extraction instrument.

* Set the elution temperature to 65°C, then follow the program settings in the table below:

Step	Column	Operation	Wait time	Mix time	Mix speed	Absorb time	Temperature
1	2/8	Transfer beads	-	10 sec	Fast	30 sec	-
2	1/7	Incubation	-	5 mins	Fast	30 sec	-
3	3/9	Wash 1	-	1 min	Fast	30 sec	-
4	4/10	Wash 2	-	1 min	Fast	30 sec	-
5	5/11	Wash 3	-	1 min	Fast	30 sec	-
6	5/11	Dry out	3 mins	-	-	-	-
7	6/12	Elution	-	5 mins	Fast	30 sec	65°C
8	2/8	Discard beads	-	5 sec	Fast	-	-

5. At the end of the program, pipette out the DNA sample from wells of column 6/12, and store at -20°C or -70°C

Common Problems and Solutions:

Common problems	Reason	Solution
Microbial DNA loss	Microbial cell loss	Use fresh sample, avoid freeze-thaw cycle
	Microbial loss during supernatant removal	When enriching samples such as biological fluids, swabs, and microbial culture and removing the supernatant, avoid touching the bottom precipitate.
	Insufficient microbial cell lysis	Strictly follow the instructions provided in the manual, or extend the mechanical disruption time appropriately.
	Residual magnetic beads	If the sample contains a high amount of lipid substances, making it viscous, you can consider extending the lysis time appropriately to ensure thorough decomposition of impurities other than nucleic acids.
Precipitates during extraction	If LB is stored at low temperature or for a prolonged period, precipitation may occur.	If precipitation appears in LB, it must be incubated at 56°C until the precipitation is completely dissolved before use.
Low extraction rate	Insufficient cell lysis or excessive enrichment of sample cell count.	Extend the disruption time appropriately and reduce the amount of sample input.

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