MGIEasy Microbiome DNA Extraction Kit User Manual

Manual Version：1.0　　Model: MD01T-96

【Product Name】

MGIEasy Microbiome DNA Extraction Kit

【Package】

Cat. No.	Model	Specification
1000027955	MD01T-96	96 preps

【Intended Use】

MGIEasy Microbiome DNA Extraction Kit can efficiently purify the microbial DNA from various samples such as serum, plasma, CSF (cerebrospinal fluid), BALF (alveolar lavage fluid), sputum, bacterial culture fluid, etc. This kit is suitable for the extraction of DNA from gram-negative bacteria, gram-positive bacteria, yeasts, DNA viruses, etc. This kit is either suitable for manual operation or automated extraction on MGISP-960（High-throughput automated sample preparation system）.

【Kit Components】

Table 1 Main Components and specifications

	Reagent	Package and amount
Components	Buffer LB	29 mL×1 bottle
	Buffer BW1	20 mL×1 bottle
	Buffer BW2	24 mL×1 bottle
	Buffer EB	20 mL×1 bottle
	Proteinase K	2 mL×1 tube
	Magnetic Beads H	2 mL×1 tube
	Buffer MRP	2 mL×1 tube

【Storage Conditions】

Proteinase K and Magnetic Beads H are recommended to be stored at 2-8 °C. The other components are stored at room temperature (15-25°C).

【 Applicable Automation Instrument 】

Applicable automation instrument:

High-throughput automated sample preparation system, Model: MGISP-960 config 1/2/6/7/8/9/10.

【 Experimental Workflow 】

Please follow the workflow as below:

A. Required Materials Not Supplied

a) Required Materials for Manual Workflow

Table 2 Required Materials for Manual Extraction

Type	Item Name	Note
Instrument	Table top centrifuge	Rotation speed not lower than 10,000 rpm/min
	Vortex	/
	Metal heater	Or instead by water bath
	1.5 mL tube magnets	/
	Pipette	1 mL, 200 μL, 20 μL
Reagent	Absolute ethanol	AR
	isopropanol	AR
Consumable	1.5 mL centrifuge tube	Nonstick, DNase-free, RNase-free
	Tips	1 mL, 200 μL, 20 μL
	50 mLtube	DNase-free, RNase-free

b) Required Materials for MGISP-960 Automatic Workflow

Table 3 Required Materials for Automatic Extraction

Type	Item Name	Note
Instrument	Plate centrifuge	/
	Vortex	/
	Pipette	1 mL, 200 μL, 20 μL
	Adapter plate (for 96-well half skirt PCR plate)	Cat. No. 010-901739-00,MGI
Reagent	Absolute ethanol	AR
	Isopropanol	AR

Consumable	Tips	1 mL, 200 μL, 20 μL
	250 μL automated filter tips	Cat. No. 1000000723, MGI
	1.3 mL U-bottom deep-well plate	Cat. No. 1000004644, MGI
	96-well half skirt PCR plate	Cat. No. 1000000671, MGI
	50 mL tube	DNase-free, RNase-free

B. Read before use

1. If Buffer LB and Buffer BW1 have precipitates, they can be re-dissolved in a 37 °C water bath, shaken and mixed well before used.
2. Replace magnetic bead buffer：If you are very concerned about background bacterial DNA contamination, the kits stored for more than 6 months need to perform this step. Place the magnetic bead tube under magnetic force, and discard the supernatant after the magnetic beads are completely adsorbed. Then add the same amount of Buffer MRP to the magnetic bead tube.
3. All reagents and samples need to equilibrate to room temperature (15-25°C) before use.
4. Before use, please make sure to add absolute ethanol into Buffer BW1 and Buffer BW2 according to the amount indicated on the reagent bottle label.
5. The components of Buffer EB are 10 mM Tris-HCl (pH 8.0) and 0.5 mM EDTA (pH 8.0). If Buffer EB is not compatible with your downstream application, you can prepare or use other elution buffer according to your needs.
6. This product is for Research Use Only and is not intended for clinical diagnosis.
7. All samples and reagents should be avoided to directly contact with skin and eyes; do not swallow; once happened, immediately rinse with plenty of water and go to the hospital for treatment in time.
8. All samples and various wastes should be disposed in accordance with relevant regulations.
9. If you have other questions, please contact MGI technical support:

   MGI-service@mgi-tech.com

C. Sample processing

1. Perform the following procedure according to the sample type.

   1) **Cerebrospinal fluid, alveolar lavage fluid , Sputum sample：**

Take 700 μL-800 μL sample into a 2.0 mL tube (500 μL zirconia beads containing), put it into the wall breaker at 5000 rpm for 1 min, centrifuge at 8000 rpm for 1 min, take 300 μL of supernatant to continue the operation.

2) Viscous sputum: choose one of two processing methods

① Take 500 μL-600 μL sample into a 2.0 mL tube (500 μL zirconia beads containing) then add 200 μL Buffer LB, put it into the wall breaker at 500 rpm for 1 min, centrifuge at 8000 rpm for 1min, take 300 μL of supernatant to continue the operation.

② Take 500μL of sample into a 2 mL tube and add 500μL of 2% NaOH. Vortex for 1 min and let stand at room temperature for 15-20 min until the sputum becomes a fluid liquid. Centrifuge at 10000 rpm for 1 min, discard the supernatant, add 300 μL PBS or NF water and vortex to mix well, then continue the operation

3) **serum, plasma sample**:

Take 300-1000 μL of sample, centrifuge at 8000 rpm for 5 min. Keep 300μL of supernatant, discard the rest of the supernatant to continue the operation.

4) **Microbial culture:**

Gram-negative bacteria: Take 1 mL of the culture in a 1.5 mL centrifuge tube, centrifuge at 8,000 rpm for 5 min, discard 700 μL of the supernatant, save 300 μL, vortex and resuspend the microbial cells, and continue the operation.

Gram-positive bacteria: Take 1 mL of the culture in a 1.5 mL centrifuge tube, centrifuge at 8,000 rpm for 5 min, discard 800 μL of the supernatant, save 200 μL, vortex and resuspend the microbial cells. Add 100 μL lysozyme (40 mg/mL), incubate at 37°C for 20 min, and continue the operation.

Fungus: Take 1 mL of the culture in a 1.5 mL centrifuge tube, centrifuge at 8,000 rpm for 5 min, discard 800 μL of the supernatant, save 200 μL, vortex and resuspend the microbial cells. Add 100 μL lysozyme (40 mg/mL) and 2 μL lysozyme solution(10u/μL), incubate at 37°C for 20 min, and continue the operation.

Note: The number of bacteria should not exceed 10^9 and the number of fungi should not exceed 10^8.

D. Manual Extraction Standard Workflow

1. Add 20 μL Proteinase K and 300 μL Buffer LB to prepared sample, mix thoroughly by vortex, and incubate at 65°C for 15 min, vortex 3 times during this period.
2. High temperature incubation (For extracting microorganisms that are difficult to lyse,

including fungi, gram-positive bacteria and candida etc.)

After step 1, continue to incubate at 90 °C for 10 min. Centrifuge at 8000 rpm for 1 min and transfer the supernatant to a new centrifuge tube.

3. Add 350 μL isopropanol, mix thoroughly by vortex.
4. Add 20 μL Magnetic Beads H, mix thoroughly by vortex, let stand at room temperature for 3 min, vortex twice during this period.
5. Place the centrifuge tube on Magnetic shelf. After the magnetic beads are completely adsorbed, carefully discard the supernatant liquid.
6. Add 500 μL Buffer BW1 (make sure to add absolute ethanol). Remove the centrifuge tube from the Magnetic shelf, mix thoroughly by vortex, and let stand at room temperature for 1 min.
7. Place the centrifuge tube on magnetic shelf. After the magnetic beads are completely adsorbed, carefully discard the supernatant liquid.
8. Add 600 μL Buffer BW2 (make sure to add absolute ethanol). Remove the centrifuge tube from the Magnetic shelf, mix thoroughly by vortex, and let stand at room temperature for 1 min.
9. Place the centrifuge tube on Magnetic shelf. After the magnetic beads are completely adsorbed, carefully discard the supernatant liquid.
10. Repeat Step 8 and 9 once.
11. Remove the centrifuge tube from the magnetic shelf and centrifuge briefly, then put the centrifuge tube back on the magnetic shelf, carefully discard the remaining liquid.
12. Keep the lid open at room temperature for 3-5 min.
13. Add 80 μL Buffer EB (adjust the re-dissolution volume as needed), mix thoroughly by vortex, and incubate at 56°C for 5 min, vortex twice during this period.
14. Place the centrifuge tube on the magnetic shelf. After the magnetic beads are completely adsorbed, carefully transfer supernatant to a new collection tube.

E. MGISP-960 Automated Extraction Standard Workflow

E.1. MGISP-960 Automated Extraction Preparation

1. Instrument Setup

1) Before first use, install application scripts according to ***MGISP-100 & MGISP-960 Application Script Installation Instructions.***

2) Perform a pre-clean after powering on the device and before experiment according to ***MGISP-100 & MGISP-960 Cleaning Instructions***.

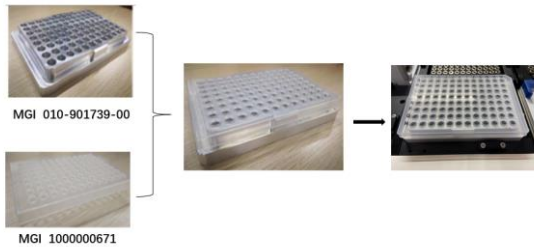
2. Preparing Consumables

Take out the consumables required for one workflow at room temperature for further use, as listed in the table 4:

Table 4 Customer-prepared Materials for MGISP-960 Automated Extraction

Consumables	Brand	Cat. No.	Quantity
250 μL automated filter tips	MGI	1000000723	6 Boxes
1.3 mL U-bottom deep-well plate	MGI	1000004644	7 Plates
96-well half skirt PCR plate	MGI	1000000671	1 Plate
Adapter plate (for 96-well half skirt PCR plate)	MGI	010-901739-00	1 Plate

Note: the usage of Adapter+96-well half skirt PCR plates as shown in the following figure (Adapter reusable) :



3. Preparing Samples

The script of MGISP-960 automation system is suitable for 1-96 sample.

According to the type of sample, the samples need to be prepared before running on MGISP-960. Take enough sample to a deep-well plate (MGI, 1000004644) so that there has 200 μL sample can be transferred (220 μL is recommend). And make sure that there are no air bubbles at the bottom and no hanging liquid on the side walls. Keep on ice for later use.

4. Preparing Reagents

1) Preparation of Buffer BW1: Absolute ethanol needs to be added according to the label.

2) Preparation of Buffer BW2: Absolute ethanol needs to be added according to the label.

3) Preparation of Buffer Mixture according to table 5, Mix upside down.

Table 5 Lysis Mix

Item	Reagent	1 rxn
Lysis Mix	Proteinase K	20 μL
	Buffer LB	200 μL

4) Take out 7 1.3 mL U-bottom deep-well plate (MGI, 1000004644) add the reagents according to the table 6.

Table 6 Reagent Volume of Sample, Lysis Mix, Buffer EB, Buffer BW1, Buffer BW2, Magnetic Beads

Reagent	Consumables	Brand	Cat. No.	Volume to add for each well
Sample	U-bottom deep-well plate	MGI	1000004644	220 μL
Prepared Lysis Mix	U-bottom deep-well plate	MGI	1000004644	220 μL
Buffer EB	U-bottom deep-well plate	MGI	1000004644	90 μL
isopropanol	U-bottom deep-well plate	MGI	1000004644	270 μL
Buffer BW1	U-bottom deep-well plate	MGI	1000004644	320 μL
Buffer BW2	U-bottom deep-well plate	MGI	1000004644	640 μL
Magnetic Beads H	U-bottom deep-well plate	MGI	1000004644	20 μL

E.2. MGISP-960 Operation

1) Double-click the icon of MGISP-960 on the desktop. The mode selection interface is displayed, as shown in following figure 1. Select "**Real**" and click "**Create**".

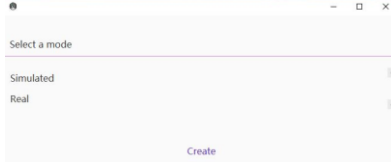


Figure 1 Mode Selection Interface

2) In the Authentication interface, click "**User Entry**" to enter the initialization interface.

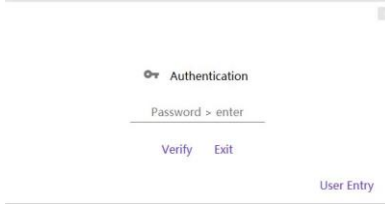


Figure 2 Authentication Interface

3) The initialization interface is displayed, as shown in following figure 3.

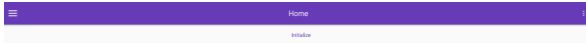


Figure 3 Initialization Interface

4) Click **"Initialize"**. The initialization takes about 2 min. If initialize successfully is displayed (as shown in following figure 4, the device is connected successfully, and you can go to the next step

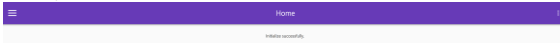


Figure 4 Initialization Successful Interface

Note: If the initialization fails, check whether the power switch is turned on, and whether more

than one software program is running. Try to restart the software. If the problem persists, contact MGI technical support.

5) Click the menu button and select "**Run Wizard**" in the menu. In the Run Wizard interface, click "**Solution**", and select **[JB-A09-098 MGISP-960 MGIEasy Microbiome DNA Extraction RV1.0_SV1.0]** , click "**Script**", to select **[MGIEasy Microbiome DNA Extraction v1.0.py]** , operation deck arrangement of the first phase is displayed, as shown in following figure 6 and table 7. Follow the on-screen instructions to place the consumables, samples, and reagents, as shown in the figure 6. Confirm the placement and close the door.

Figure 5 Run Wizard Interface

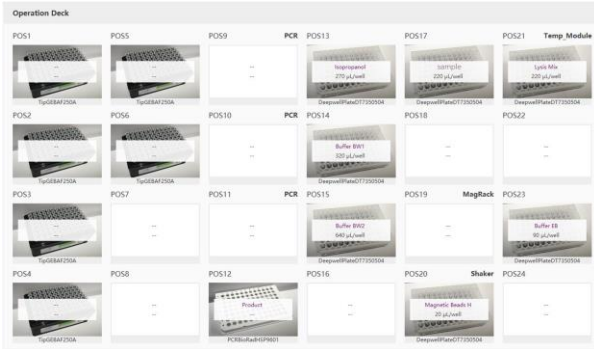


Figure 6 Operation Deck Arrangement

Table 7 Operation Deck Arrangement

Name	Position
250 μL automated filter tips	Pos1-Pos6
96-well half skirt PCR plate + Adapter plate	Pos12
isopropanol	Pos13
Buffer BW1	Pos14

Buffer BW2	Pos15
Sample	Pos17
Magnetic Beads H	POS20
Lysis Mix	Pos21
Buffer EB	Pos23

6) Click "**Run**" to start extraction workflow.

7) It is expected to run 1 h 10 min. After the process is finished, the product at Pos12 is taken out.

8) Perform the next testing operation.

9) Dispose of the used deep-well plates, PCR plates, and waste bag to the designated waste area. Perform a post-clean before powering off the device according to ***MGISP-100 & MGISP-960 Cleaning Instructions***.

【 Precautions 】

1. This product is only used for scientific research, not for clinical diagnosis, please read this instruction carefully before use.
2. Please familiarize the operation and precautions of various instruments to be used before testing.
3. When all the reagents are taken out from the specified storage environment, please use them according to the requirements. The reagents should be shaken and mixed before use.
4. The micro- Pipette should be used for sample addition.
5. All samples and reagents should be avoided to directly contact with skin and eyes; do not swallow, once happen, immediately rinse with plenty of water and go to the hospital for treatment in time.
6. All samples and various wastes should be treated in accordance with relevant regulations.

【 Production Company Information 】

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