MGIEasy Stool Microbiome DNA Extraction Kit User Manual

Manual Version: 3.0 Model: SD02T-96, SD02T-384

【Product Name】

MGIEasy Stool Microbiome DNA Extraction Kit

【Package】

Cat. No.	Model	Specification
940-000122-00	SD02T-96	96 preps
940-000123-00	SD02T-384	384 preps

【Intended Use】

Used for human stool microbiome DNA extraction, enrichment, purification.

【Inspection Principle】

MGIEasy Stool Microbiome DNA Extraction Kit can be used to extract and purify microbial genomic DNA from fresh or frozen human stool samples. This product uses superparamagnetic nano magnetic bead capture technology and unique impurity removal technology to effectively remove impurities in the samples and obtain high-quality, high-purity genomic DNA. The extracted genomic DNA can be used in a variety of routine operations, including enzyme digestion, PCR, fluorescent quantitative PCR, library preparation, microarray hybridization, and high-throughput sequencing.

【Kit Components】

Table 1 Main components and specification

Reagent	Package and amount	
	(96 Preps)	(384 Preps)
PCB	70 mL×1 bottle	280 mL×1 bottle
PLB-M	50 mL×1 bottle	200 mL×1 bottle
PFB-M	12.5 mL×1 bottle	50 mL×1 bottle
PW1-M	20 mL×1 bottle	80 mL×1 bottle
PW2	24 mL×1 bottle	96 mL×1 bottle
PB	15 mL×1 bottle	60 mL×1 bottle
Magnetic Beads-T	2 mL×1 tube	8 mL×1 bottle
Proteinase K	1 mL×1 tube	4 mL×1 bottle

【Storage Conditions】

The product can be stored at 2°C to 30°C for 12 months.

Note:

1. **All Components can be transported and stored at 2°C to 30°C.**
2. **When proteinase K is precipitated, proteinase K needs to be replaced. It is normal for other reagents to have a small amount of crystal precipitation, which does not affect the product performance.**

【Applicable Automation Instrument】

Applicable automation instrument:

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

Automated Nucleic Acid Extraction and Purification System, Model: MGISP-NE384.

【Sample Conditions】

1. This kit is designed to extract DNA from stool samples. For fresh stool samples, collect them within 2 h and store them in the preservation solution at room temperature for 7 days or at -80°C for 1 year. The collected samples can also be placed directly in a -80°C refrigerator or dry ice, stored for less than 1 year. If the samples are not collected and used within 2 h, the microorganisms in the stool will die and release a large amount of nuclease, which may degrade the extracted genomic DNA and lead to a low yield; as a result, it is difficult to guarantee the integrity of the extracted genomic DNA.

2. The fresh stool samples can be stored at 4°C temporarily, and the extraction experiment should be completed at the same day. If the fresh stool samples are not used immediately, the samples need to be stored following the preservation method in Condition 1. Avoid repeated freezing and thawing, and fecal storage solution should be added when the samples are thawed. For example，we add 8mL storage buffer to a tube with 2 grams feces sample. After the samples are evenly mixed, use a broad pipette tip to transfer 3 to 5 mL the suspension into a 5 mL centrifuge tube, and place it at -80°C. When sample extraction is required, take out one tube each time.

3. Sample transportation: The samples put in the fecal preservation solution can be transported at room temperature for a period of less than 7 days. The samples put in the sampling cup should be transported on dry ice, and they should not be transported for a period longer than 7 days. Avoid repeated freezing and thawing during transportation.

4. Sample Safety: All samples are regarded as potentially infectious items and shall be handled in accordance with relevant national standards.

【Experimental Workflow】

Please follow the workflow below:

A. Required Materials Not Supplied

a) Required Materials for Manual Workflow

Table 2 Required materials for manual extraction

Type	Item Name	Note
Instrument	Desktop centrifuge	Centrifuge Speed≥ 12,000 rpm
	Vortexer	Centrifuge Speed≥ 2,500 rpm
	Thermomixer	/
	1.5 mL magnetic rack	/
	Pipette	1 mL, 200 μL, 20 μL
Reagent	Absolute ethanol	AR
	Isopropanol	AR
	MGIEasy Stool Sample Collection Kit	Item: 1000003702(Standard version)
	Grinding beads	MGI: 940-000136-00
Consumable	1.5 mL, 2.0 mL centrifuge tube	Nonstick, DNase-free, RNase-free
	Tips	1 mL, 200 μL, 20 μL

b) Required Materials for Automatic Workflow:

Table 3 Required materials for MGISP-960

Type	Name	Brand	Item
Instrument	Vortexer (Maximum Centrifuge Speed≥ 2,500 rpm)	/	/
	Desktop centrifuge (Maximum Centrifuge Speed≥ 12,000 rpm)	/	/
	Thermomixer	/	/
	Pipette	/	/
Reagent	Absolute ethanol (AR)	/	/
	Isopropanol (AR)	/	/
	MGIEasy Stool Sample Collection Kit	MGI	1000003702
	Grinding beads	MGI	940-000136-00
Consumable	Tips (1 mL, 200 μL, 20 μL)	/	/
	1.5 mL, 2.0 mL centrifuge tube (Nonstick, DNase-free, RNase-free)	/	/
	250 μL automated filter tips	MGI	1000000723
	1.3 mL U-bottom deep-well plate	MGI	1000004644
	Adapters (for Half-skirted 96-well PCR plate)	MGI	010-901739-00
	Half-skirted 96-well PCR plate	MGI	1000000671

Table 4 Required materials for MGISP-NE384

Type	Name	Brand	Item
Instrument	Vortexer(Maximum Centrifuge Speed≥ 2,500 rpm)	/	/
	Desktop centrifuge (Maximum Centrifuge Speed≥ 12,000 rpm)	/	/
	Thermomixer	/	/
	Pipette	/	/
Reagent	Absolute ethanol	/	/
	Isopropanol	/	/
	MGIEasy Stool Sample Collection Kit	MGI	1000003702
	Grinding beads (in countries except China)	MGI	940-000136-00
Consumable	Tips (1 mL, 200 µL, 20 µL)	/	/
	1.5 mL, 2.0 mL centrifuge tube (Nonstick, DNase-free, RNase-free)	/	/
	96-well tips comb	MGI	1000025661
	2.2 mL V-bottom deep-well plate	MGI	1000008088

B. Read before use

1. Avoid repeatedly freezing and thawing samples, which may result in low DNA quality.
2. All reagents and samples need to be equilibrated to room temperature (10 °C to 30 °C) before use.
3. Before use, please make sure to add absolute ethanol (100%) into Buffer PCB, PW1-M and PW2 according to the amount indicated on the reagent bottle label. Make sure to add isopropanol according to the amount indicated on the reagent bottle label.
4. Please use the recommended consumables for automated or manual operations.
5. Please read the manual carefully before the experiment.
6. Buffer PB reagent component is 10 mM Tris-HCl (pH8.0).

C. Sample Pretreatment

1. **Solid or semi-solid samples**: Weigh out 180 mg to 220 mg of stool sample into a 2.0 mL

centrifuge tube, and add 1 mL of fecal preservation solution/PBS into the tube. Adjust the vortex mixer to the maximum (at least 2500 rpm), shake and mix for 3 to 5 minutes until the solution completely changes color and the sample is evenly suspended. Then place the tube on the tube rack and allow it to stand for 5 minutes. Use 1 mL pipet to transfer 200μL to 500 μL of the upper solution to a new 2.0 mL centrifuge tube (if there are impurities blocking the pipette tip, the front part of the tip can be cut off as appropriate).

Sample in stool preservation solution: Shake and mix well so that the sample is evenly suspended. Transfer 200 μL to 500 μL of suspension to a new 2.0 mL centrifuge tube (if there are impurities blocking the pipette tip, the front part of the tip can be cut off as appropriate).

2. Add 1000 μL Buffer PCB (ensure that absolute ethanol has been added) to the 2.0 mL centrifuge tube with fecal suspension. Vortex at maximum (at least 2500rpm) for 15 seconds.
3. Centrifuge at 12000rpm for 2 minutes at room temperature, discard all supernatant (avoid pipetting the solid sediment).
4. Add 10 μL of proteinase K, 500 μL of PLB-M and grinding beads (add beads to 100μL scale line of a clean 1.5mL centrifuge tube or weigh the beads to 0.15 grams) into the tube with sample suspension. Vortex at the maximum speed for 60 seconds (For more Gram-positive bacteria, grinding machine, brand: Mobio/QIAGEN, recommended parameters: 30Hz/1800rpm grinding for 5 minutes). After grinding and mixing, place the centrifuge tube on a thermomixer, set the temperature at 70 °C and the speed at 1000 rpm, and incubate for 20 minutes.
5. After incubation is completed, take out the centrifuge tube. Centrifuge at 12000rpm for 2 minutes at room temperature and pipette 400 μL of supernatant into a new 1.5 mL centrifuge tube.

D. Manual Extraction Standard Workflow

1. Add 500 μL of PFB-M (ensure that isopropanol has been added) and 20 μL of Magnetic Beads-T to step C.4 tube, mix thoroughly, incubate at room temperature for 2 minutes, and mix once or twice during the process by vortexing for 3 seconds.

Note: Magnetic Beads-T stand at room temperature for 30 minutes beforehand. Vortex and mix thoroughly before use.

2. Briefly centrifuge the tube and place it on the magnetic rack for 2 minutes. When the liquid is clear, carefully remove and discard the supernatant.
3. Remove the tube from the magnetic rack. Add 500 μL of PW1-M (ensure that absolute ethanol has been added), and mix thoroughly for 1 minute.

Note: After adding PW1-M, please mix thoroughly, otherwise the purity of nucleic acid extracted will be affected.

4. Briefly centrifuge the tube and place it on the magnetic rack for 1 minute. When the liquid is clear, carefully remove and discard the supernatant.
5. Remove the tube from the magnetic rack. Add 600 μL of PW2 (ensure that absolute ethanol has been added), and mix thoroughly for 1 minute.
6. Briefly centrifuge the tube and place it on the magnetic rack for 1 minute. When the liquid is clear, carefully remove and discard the supernatant.
7. Repeat step 5 to step 6 again, aspirate the remaining liquid in the centrifuge tube as much as possible.
8. Open the tube, and dry at room temperature for 5 minutes to ensure that the ethanol completely evaporates.
9. Remove the tube from the magnetic rack. Add 100 μL to 150 μL of Buffer PB, mix by vortex and place it on a thermomixer. Incubate at 56 °C, 1000 rpm for 5 minutes.
10. Centrifuge briefly and place the centrifuge tube on the magnetic rack. When the liquid is completely clear, carefully transfer the supernatant to a new 1.5 mL tube. Label and store the solution at -20 °C.

E. MGISP-960 Automated Extraction Standard Workflow

E1. MGISP-960 Automated Extraction Preparation

1. Instrument Preparing

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

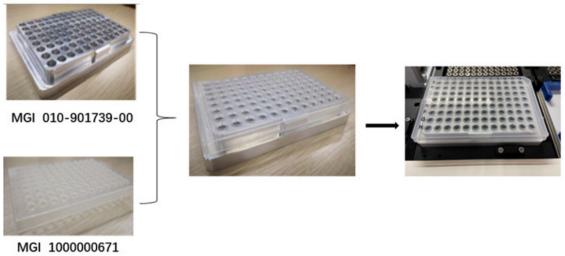
2) Perform a pre-clean after powering on the device and before the experiment according to ***MGISP-100 & MGISP-960 Cleaning Instructions***.

2. Preparing Consumables

Take out the consumables required for one workflow at room temperature for further use, as listed in the table 5:

Table 5 Material required but not provided

Consumables	Brand	Cat. No.	Quantity
250 μL automated filter tips	MGI	1000000723	6 Boxes
1.3 mL U-bottom deep-well plate	MGI	1000004644	5 Plates
Half-skirted 96-well PCR plate	MGI	1000000671	1 Plate
Adapter (for half-skirted 96-well PCR plate)	MGI	010-901739-00	1 Plate



Note: the usage of Adapter+96-well half skirt PCR plates is shown in the upper figure (Adapter reusable), and it can directly replace the Hard-shell thin-well skirted PCR plates, white shell/clear well (MGI, 1000012059).

Note: For Configuration 1/2/6/7/8/10-MGISP-960, adapter (MGI,010-901739-00) needs to be purchased.

3. Preparing Samples

1) The script of MGISP-960 automation system is suitable for 96 samples at one time.

2) Refer to ***C. Sample Pretreatment*** to complete the pretreatment of stool samples.

3) Take out a deep well plate (MGI, 1000004644) and marked it as Sample, add 240 μL/channel of supernatant to each well. And make sure that there are no air bubbles at the bottom and no hanging liquid on the side walls. Keep it on ice for further use.

4. Preparing Reagents

1) For one reaction, every 20 μL Magnetic Beads-T are mixed with 300 μL PFB-M, and mix them thoroughly。

2) Take out 5 U-bottom deep-well plates (MGI, 1000004644), and mark them as **【PFB-M+Magnetic beads-T】**, **【PW1-M】**, **【PW2】**, and **【PB】** respectively. Add reagents according to Table 6.

Table 6 Input volume of PFB-M, PW1-M, PW2, Magnetic Beads-T, and PB

Item	Plate name	Volume/Well
(PFB-M+ Magnetic beads -T) + Sample	(PFB -M +Magnetic beads-T mixed liquor) +sample	320 μL (PFB -M +Magnetic beads-T mixed liquor) +240 μL sample
PW1-M	PW1-M	400 μL
PW2	PW2	800 μL
PB	PB	120 μL

Note: Mix Magnetic Beads-T thoroughly before use.

E2. MGISP-960 Operation

1. Instrument 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.

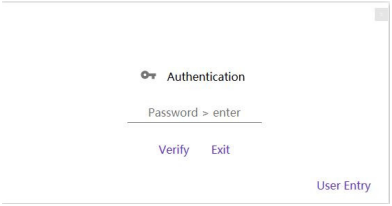


Figure 2 Authentication interface

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

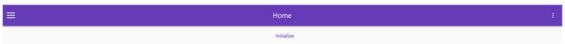


Figure 3 Initialization interface

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

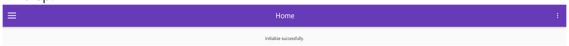


Figure 4 Successful initialization interface

Note: If the initialization fails, check whether the power switch is turned on, and whether more than one software program is running. Try restarting 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-A09-120 MGIEasy Stool Microbiome DNA Extraction RV1.0_SV1.0】, click 【Script】, to select 【Microbiome Genomic DNA Extraction for Stool_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.

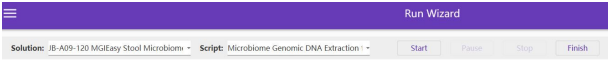


Figure 5 Run wizard interface

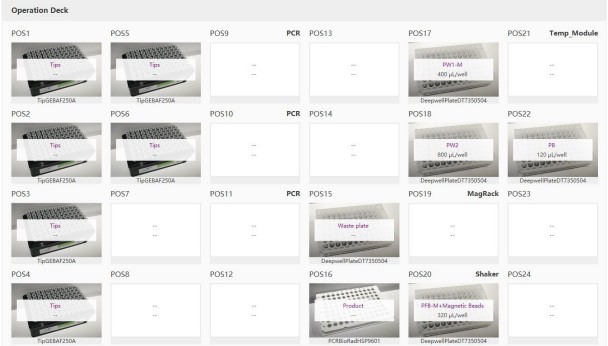


Figure 6 First phase operation desk arrangement

Table 7 First phase operation deck arrangement

Name	Position
250 μL automated filter tips	Pos1-Pos6
Waste plate	Pos15
Adapter+ Half skirt 96-wel PCR plate	Pos16
PW1-M	Pos17
PW2	Pos18
PFB-M+Magnetic Beads-T	Pos20
PB	Pos22

6) Click 【**Run**】 to start extraction workflow.

7) It is expected to run 1 hour, and you can pause or resume the workflow if necessary. Take out the product at Pos16 after the process finished.

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***.

✔ **Stopping point: The extracted DNA can be stored at -20 °C.**

F. MGISP-NE384 Automated Extraction Standard Workflow

F1. MGISP-NE384 Automated Extraction Preparation

1. Preparing Device

1) Before first use, please confirm that the application script has been imported into the location of MGISP-NE384. For example, C:/MGISP-NE384/Scripts/MGIEasy Microbiome DNA Extraction for Stool.mgi

2) Before starting each round of experiment, please make sure that the machine has finished **【Clean】**.

2. Preparing Consumable

Take out the consumables required for one workflow for 384 samples, as listed in the table below:

Table 8 Materials required but not provided

Consumables	Brand	Cat. No.	Quantity
96 well tips comb	MGI	1000025661	4 pieces
2.2 mL V-bottom deep-well plate	MGI	1000008088	24 plates

3. Preparing Samples

1) The Automated Nucleic Acid Extractor can process 1 to 384 samples at one time.

2) Refer to ***C. Sample Pretreatment*** to complete the pretreatment of stool samples.

3) Take out a 2.2 mL V-bottom deep well plate (MGI, 1000008088) and mark it as **【Sample supernatant + PFB-M】**, add 400 µL of supernatant to each well. And make sure that there are no air bubbles at the bottom and no hanging liquid on the side walls. Keep on ice for further use.

4. Preparing Reagents

1) According to the number of samples, transfer the extraction reagents into new 2.2 mL V-bottom deep-well plates.

2) Magnetic Beads-T need to be diluted. For one reaction, every 20 µL Magnetic Beads-T are mixed with 280 µL MiliQ water or Nuclease-Free water, and mix them thoroughly.

3) MGISP-NE384 can match 1 to 4 Lanes 96 samples extraction. In addition to the deep-well plate **【Sample supernatant +PFB-M】,** Each Lane requires 5 deep-well plates (MGI, 1000008088), marked them as **【Diluted Magnetic Beads-T】**, **【PW1-M】**, **【PW2-1】**, **【PW2-2】** and

【PB】. Add reagents according to Table 9.

Table 9 Input volume of each set of reagents

Item	Reagent	Volume/well
Sample supernatant + PFB-M	Sample supernatant and PFB-M	400 μL Sample supernatant + 500 μL PFB-M
Diluted Magnetic Beads-T	Diluted Magnetic Beads-T	300 μL
PW1-M	PW1-M	500 μL
PW2-1	PW2	600 μL
PW2-2	PW2	600 μL
PB	PB	100 μL to 150 μL

F2.MGISP-NE384 Operation

1. Instrument Operation

1) Import MGISP-NE384 script according to the path specified by MGI instrument after-sales engineer.

2) Double-click the icon of MGISP-NE384 on the desktop. The authentication interface will be displayed. Select 【**User**】, enter the password 【**123456**】, and click 【**login**】.

3) The initialization interface will be displayed.

4) Click 【**Initialize**】. The initialization takes approximately 1 minutes. If 【**Initialized**】 is successfully displayed, it means that the device is connected successfully, and you can go to the next step.

Note: If the initialization fails, check whether the power switch is turned on, and whether more than one software program is running. If yes, please restart the software. If the problem persists, please contact MGI technical support.

5) Select the【**Clean**】 option, empty the console, wipe the console and tray with a dust-free paper soaked with 75% alcohol and close the window. Click【**Start**】, and the instrument will open the fan filter unit and UV lamp to clean the internal environment of the instrument. The default cleaning time is 20 minutes. You can also adjust the cleaning time accordingly.

6) After【**Clean**】, return to the main interface and select 【**Workflow**】.

7) In the Workflow interface, click **【Script】**, select **【MGIEasy Microbiome DNA Extraction for Stool.mgi】**. Follow the on-screen instructions to place the consumables and reagents, as shown in Table10. Install the tips comb.

Note: If only one or more lanes of Lane A, Lane B, Lane C, Lane D are used, please place the different reagent plates in the corresponding position of the same lane according to Table 10, and select the corresponding lane for experiment.

Table 10 Operation deck layout

Reagent plates	Position
Sample supernatant + Isopropanol	LaneA, LaneB, LaneC, LaneD: Pos1
Diluted Magnetic Beads-T	LaneA, LaneB, LaneC, LaneD: Pos2
PW1-M	LaneA, LaneB, LaneC, LaneD: Pos3
PW2-1	LaneA, LaneB, LaneC, LaneD: Pos4
PW2-2	LaneA, LaneB, LaneC, LaneD: Pos5
PB	LaneA, LaneB, LaneC, LaneD: Pos6

8) Confirm that the consumables and reagents are placed correctly, and close the instrument window. Click **【Run】**. Check the corresponding test channel according to the number of samples and check if the tips comb is placed correctly. Click the **【Confirm】**.

9) The whole run takes approximately 30 minutes. Please arrange the following work properly.

10) After the run ends, please take out the extraction product at pos6 immediately. It can be used directly for subsequent experiments or stored at -20 °C.

11) Dispose of the used deep-well plates and tips comb. Select the **【Clean】** option, empty the console, wipe the console and tray with a dust-free paper soaked with 75% ethanol and close the window. Click **【Start】**, and the instrument will open the fan filter unit and UV lamp to clean the internal environment of the instrument. The default cleaning time is 20 minutes. You can also adjust the cleaning time as needed.

Note: After the experiment, please take out the extracted product immediately. It is forbidden to leave the product at Pos6 for a long time, otherwise it will affect the quality of the product.

✔ **Stopping point: The extracted DNA can be stored in the -20 °C refrigerator.**

【Precautions】

1. This product is for scientific research only, not for clinical diagnosis. Please read this instruction carefully before use.
2. Please familiarize yourself with 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. A micropipette should be used for sample addition.
5. Keep your skin and eyes from direct contact with any sample or reagent. (Do not swallow any sample or reagent.) If it happens, 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.

【Manufacturer Information】

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