MGIEasy Stool Human DNA Extraction Kit User Manual

Manual Version: 2.0 Model: SD01T-96, SD01T-1536

【Product Name】

MGIEasy Stool Human DNA Extraction Kit

【Package】

Cat. No.	Model	Specification
1000028534	SD01T-96	96 preps
1000028535	SD01T-1536	1536 preps

【Intended Use】

Used for stool human DNA extraction, enrichment, purification.

【Inspection principle】

MGIEasy Stool Human DNA Extraction Kit can be used to extract and purify 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）	（1536 Preps）
PLB	29 mL×1 bottle	461 mL×1 bottle
PHB	10 mL×1 bottle	154 mL×1 bottle
PW1	15 mL×1 bottle	231 mL×1 bottle
PW2	24 mL×1 bottle	185 mL×2 bottle
PB	15 mL×1 bottle	231 mL×1 bottle
Proteinase K	1 mL×1 tube	16 mL×1 bottle
Magnetic Beads-T	2 mL×1 tube	31 mL×1 bottle

【Storage Conditions】

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

Note:

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: 1/2/6/7/8/9/10-MGISP-960.

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

【Sample Conditions】

1. This kit is suitable 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 -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 on 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. After the samples are evenly mixed, use a broad pipette 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 a single tube each time.
3. Sample transportation: The samples put in the fecal preservation solution can be transported at room temperature for a period longer 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	≥ 12,000 rpm/min
	Vortexer	/
	Thermomixer	Or water/metal bath with shaker
	1.5 mL tube magnets	/
	Pipette	1 mL, 200 μL, 20 μL
Reagent	Absolute ethanol	AR
	Isopropanol	AR
	Fecal preservation solution (Main component is EDTA-2Na, sodium citrate, et al, used to inhibit enzyme reaction) or 1X PBS	/
Consumable	1.5 mL centrifuge tube	Nonstick, DNase-free, RNase-free
	Tips	1 mL, 200 μL, 20 μL
	50 mL centrifuge tube	No DNase, No RNase

b) Required Materials for Automatic Workflow:

Table 3 Required materials for MGISP-960

Type	Name	Brand	Item
Instrument	Vortexer	/	/
	Plate centrifuge	/	/
	Thermomixer	/	/
	Pipette	/	/
Reagent	Absolute ethanol (AR)	/	/

	Isopropanol (AR)	/	/
	Fecal preservation solution (Main component is EDTA-2Na, sodium citrate, et al, used to inhibit enzyme reaction) or 1X PBS	/	/
Consumable	Tips	/	/
	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
	50 mL centrifuge tube	/	/

Table 4 Required materials for MGISP-NE384

Type	Name	Brand	Item
Instrument	Vortexer	/	/
	Plate centrifuge	/	/
	Pipette	/	/
	Thermomixer	/	/
Reagent	Absolute ethanol	/	/
	Isopropanol	/	/
	Fecal preservation solution (Main component is EDTA-2Na, sodium citrate, et al, used to inhibit enzyme reaction) or 1X PBS	/	/
Consumable	Tips	/	/
	96-well tips comb	MGI	1000025661
	2.2 mL V-bottom deep-well plate	MGI	1000008088
	96-well PCR plate	/	/

B. Read before use

1. Avoid repeatedly freezing and thawing samples, which may result in low DNA quality.
2. If PLB or PW1 has undergone precipitation, it can be re-dissolved in a 37 °C water bath. Shake and mix well before use.

3. All reagents and samples need to be equilibrated to room temperature (10°C to 30°C) before use.

4. Before use, please make sure to add absolute (100%) ethanol into Buffer PW1 and PW2 according to the amount indicated on the reagent bottle label.

5. Please use the recommended consumables for automated or manual operations.

6. Please read the manual carefully before the experiment.

7. Buffer PB reagent component is 10 mM Tris-HCl (pH8.0). If there is a special need, prepare your own elution buffer.

C. Sample Pretreatment

1. **Solid or semi-solid samples**: Weigh out 180 mg to 220 mg of stool sample into a 1.5 mL centrifuge tube, and add 1 mL of fecal preservation solution/PBS into the tube. Adjust the vortex mixer to the maximum, shake and mix for 3 to 5 min until the solution completely changes color and the sample is evenly suspended. Then place the tube on the tube rack and wait for 5 minutes. Use 1 mL pipet to transfer 250 μL of the upper solution to a new 1.5 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. Let the tube stand for 5 minutes, and transfer 250 μL of upper solution to a new 1.5 mL centrifuge tube (if there are impurities blocking the pipette tip, the front part of the tip can be cut off as appropriate).

2. Take out a new 1.5 mL centrifuge tube, and add 10 μL of proteinase K, 300 μL of PLB, 200 μL of upper solution sample in C.1. Vortex at the maximum speed for 15 s. Place the centrifuge tube on a constant temperature mixer, set the temperature at 70 °C and the speed at 1000 rpm, and incubate for 15 min.

3. After incubation is completed, take out the centrifuge tube, centrifuge briefly, add 100 μL of PHB to the centrifuge tube, and shake well to mix.

4. Centrifuge at 12000 rpm for 2 min, take out 500 μL of supernatant and transfer it to a new 1.5 mL centrifuge tube.

D. Manual Extraction Standard Workflow

1. Add 380 μL of Isopropanol and 20 μL of Magnetic Beads-T to step C.4 tube, mix thoroughly,

incubate at room temperature for 2 min, and mix once or twice during the process by vortexing for 3 sec.

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

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

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

4. Briefly centrifuge the tube and place it on the magnetic stand for 1 min. When the liquid is clear, carefully remove and discard the supernatant.
5. Remove the tube from the magnetic stand. Add 600 μL of PW2 (ensure that absolute ethanol has been added), and mix thoroughly for 1 min.
6. Briefly centrifuge the tube and place it on the magnetic stand for 1 min. When the liquid is clear, carefully remove and discard the supernatant.
7. Perform Step 5 and 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 min to ensure that the ethanol completely evaporates.
9. Remove the tube from the magnetic stand. 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 min.
10. Centrifuge briefly and place the centrifuge tube on the magnetic stand. When the liquid is completely clear, carefully transfer the supernatant to a new 1.5 mL tube. Label and store at -20°C.

E. MGISP-960 Automated Extraction Standard Workflow

E1. 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***.

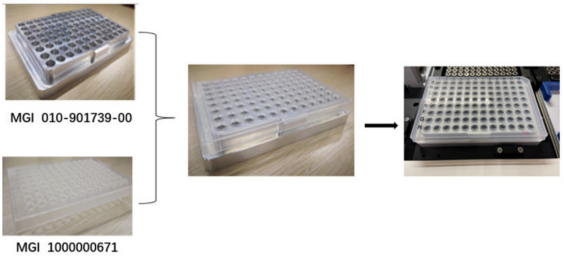
2) Perform a pre-clean after powering on the device and before the experiment according to ***MGISP-100 & MGISP-960 Cleaning Instructions***.

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	6 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 as shown in the upper figure (Adapter reusable), can directly replace the Hard-shell thin-well skirted PCR plates, white shell/clear well (MGI, 1000012059).

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

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), add 320 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 on ice for further use.

4. Preparing Reagents

1) Preparing Buffer PW1: Add absolute ethanol into the solution according to the label on the cartridge (add only before the first use).

2) Preparing Buffer PW1: Add absolute ethanol into the solution according to the label on the cartridge (add only before the first use).

3) Take out 5 U-bottom deep-well plates (MGI，1000004644), and mark them as "**Isopropanol**", "**PW1**", "**PW2**", "**Magnetic Beads-T**" and "**PB**". Add reagents according to Table 6.

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

Item	Volume/Well
Isopropanol	250 μL
PW1	320 μL
PW2	640 μL
Magnetic Beads-T	20 μL
PB	160 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.

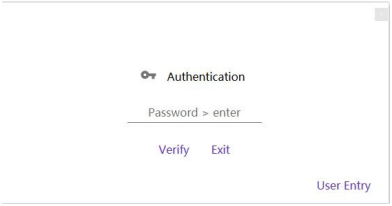


Figure 2 Authentication interface

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

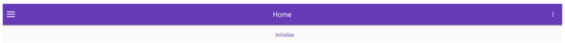


Figure 3 Initialization interface

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.

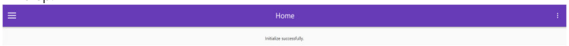


Figure 4 Successful initialization interface

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-A09-107 MGIEasy Stool Human DNA Extraction RV1.0_ S V1.0**", click "**Script**", to select "**Human 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.

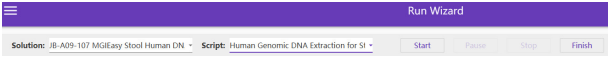


Figure 5 Run wizard interface

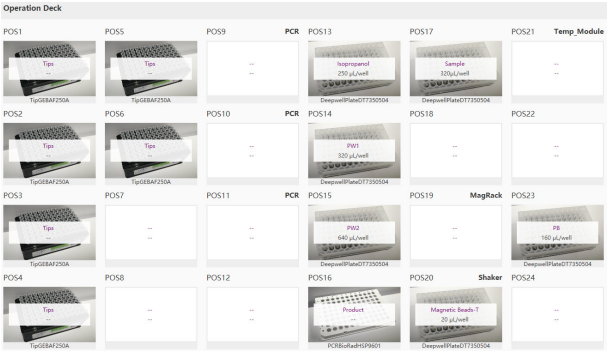


Figure 6 First phase operation deck arrangement

Table 7 First phase operation deck arrangement

Name	Position
250 µL automated filter tips	Pos1-Pos6
Isopropanol	Pos13
PW1	Pos14
PW2	Pos15
Adapter+ Half skirt 96-wel PCR plate	Pos16
Sample plate	Pos17

Magnetic Beads-T	Pos20
PB	Pos23

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

7) It is expected to run 1 h, and you can pause or resume the workflow if necessary. After the process is finished, the product at Pos16 can be 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***.

✔ **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/JB-A12-002 MGIEasy Human 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 + Isopropanol**", add 500 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, each 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 + Isopropanol",** Each Lane requires 5 deep-well plates (MGI,

1000008088), marked them as "**Diluted Magnetic Beads-T**", "**PW1**", "**PW2-1**", **"PW2-2"** and **"PB"**. Add reagents according to Table 9.

Table 9 Input volume of each set of reagents

Item	Volume/well
Sample supernatant + Isopropanol	500 μL Sample supernatant + 380 μL Isopropanol
Diluted Magnetic Beads-T	300 μL
PW1	500 μL
PW2-1	600 μL
PW2-2	600 μL
PB	100 μL to 150 μL

F2. MGISP-NE384 Operation

1. Instrument Operation

1) Double-click the icon of MGISP-NE384 on the desktop. The authentication interface will be displayed. Select "**User**", enter the password "**mgispx**", and click "**login**".

2) The initialization interface will be displayed.

3) Click "**Initialize**". The initialization takes approximately 1 minutes. If "**Initialized**" is successfully displayed, it means that the device 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.

4) 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.

5) After "**Clean**", return to the main interface and select "**Workflow**".

6) In the Workflow interface, click "**Script**", select "**JB-A12-002 MGIEasy Human DNA Extraction for Stool.mgi**". Follow the on-screen instructions to place the consumables and reagents, as shown in Table11. 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	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

7) 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**".

8) The whole run takes approximately 35 minutes. Please arrange the following work properly.

9) 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.

10) 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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