Part No.: H-020-001047-00

MGIEasy

Pathogen Microbiome DNA&RNA Extraction Kit

Instructions for Use

Version: 1.0

Leading Life Science Innovation

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Research Use Only

Wuhan MGI Tech Co., Ltd.

About the instructions for use

This instructions for use is applicable to MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit. The version of the instructions for use is 1.0 and the kit version is 1.0.

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Revision history

Version	Date	Description
1.0	September 10, 2024	Initial release

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Chapter 1 Introduction

1.1 Product name

MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit

1.2 Specifications

Set name	Model	Cat. No.	Specification
MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (48 RXN)	PMT-48	940-002223-00	48 Preps
MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (96 RXN)	PMT-96	940-002222-00	96 Preps

1.3 Intended use

This product is used to extract DNA and RNA of pathogenic microorganisms such as bacteria (including G^+ and G^- bacteria), fungi, chlamydia, mycoplasma, and DNA or RNA viruses from samples including blood (whole blood, serum, plasma), sterile body fluids (urine, sputum, cerebrospinal fluid, bronchoalveolar lavage fluid), swabs (throat, oral, nasal), stool, and tissues. The extracted products are suitable for downstream applications such as PCR, qPCR, and gene sequencing (mNGS, tNGS, RNA-seq), meeting the needs for nucleic acid extraction of pathogenic microorganisms in various applications.

1.4 Working principle

This product utilizes specific, high-binding, superparamagnetic nanomagnetic beads for the rapid extraction of high-quality DNA and RNA from pathogenic microorganisms such as bacteria (including G^+ and G^- bacteria), fungi, chlamydia, mycoplasma, and DNA or RNA viruses from samples including blood (whole blood, serum, plasma), sterile body fluids (urine, sputum, cerebrospinal fluid, bronchoalveolar lavage fluid), swabs (throat, oral, nasal), stool, and tissues.

1.5 Main components

Table 1 MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (48 RXN) Cat. No.: 940-002223-00

Name	Component	Specification	Storage condition	Validity period	Transportation condition
MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (48 RXN) Cat. No.: 940-002223-00	Buffer PML	20 mL/tube×1	2 ℃ to 30 ℃	18 months	2 ℃ to 30 ℃
	Buffer WB I	52 mL/tube×1			
	Buffer WB II	18 mL/tube×1			
	Nuclease-free water	9 mL/tube×1			
	Proteinase K	2 mL/tube×1			
	Magnetic Beads T	3 mL/tube×1			
	Grinding tube	48 preps/bag×1			

Table 2 MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (96 RXN) Cat. No.: 940-002222-00

Name	Component	Specification	Storage condition	Validity period	Transportation condition
MGIEasy Pathogen Microbiome DNA&RNA Extraction Kit (96 RXN) Cat. No.: 940-002222-00	Buffer PML	39 mL/tube×1	2 ℃ to 30 ℃	18 months	2 ℃ to 30 ℃
	Buffer WB I	104 mL/tube×1			
	Buffer WB II	41 mL/tube×1			
	Nuclease-free water	15 mL/tube×1			
	Proteinase K	4 mL/tube×1			
	Magnetic Beads T	6 mL/tube×1			
	Grinding tube	48 preps/bag×2			

Chapter 2 Applicable device

MGISP-NE32RS Automated Nucleic Acid Extractor

Chapter 3 Sample requirements

3.1 Applicable sample

This product is applicable to samples including blood (whole blood, serum, plasma), sterile body fluids (urine, sputum, cerebrospinal fluid, bronchoalveolar lavage fluid), swabs (throat, oral, nasal), stool, and tissue. It can be used to extract pathogenic microorganisms including bacteria (including G^+ and G^- bacteria), fungi, chlamydia, mycoplasma, and DNA&RNA viruses.

3.2 Sample amount requirements

Sample type		Sample volume	
		Manual extraction	Extraction on MGISP-NE32RS
Blood	Whole blood	50 µL to 100 µL	50 µL to 80 µL
	Serum	200 µL	200 µL
	Plasma	200 µL	200 µL
Sterile body fluids	Urine	200 µL	200 µL
	Sputum	10 µL to 50 µL	10 µL to 50 µL
	Cerebrospinal fluid	200 µL	200 µL
	Bronchoalveolar lavage fluid	200 µL	200 µL
Swabs (throat, oral, nasal)		200 µL	200 µL
Stool	Fresh or frozen stool	2 mg to 10 mg	1 mg to 4 mg
	Stool stored with stool preservative	8 µL to 40 µL	4 µL to 16 µL
Tissue		5 mg to 50 mg	5 mg to 20 mg

3.3 Sample storage

- If the freshly collected samples will be used within 24 hours, store them at 2 ℃ to 8 ℃ .

- If the freshly collected samples cannot be used within 24 hours, store them in a -20 ℃ freezer temporarily or -80 ℃ and below for a long time. Do not freeze and thaw frozen samples frequently during storage.

  Tips Do not freeze and thaw frozen samples frequently. Otherwise, the DNA quality may decrease.

3.4 Sample transportation

- For samples stored with stool preservative, transport them at room temperature for up to 7 days.
- For other samples, use the dry ice for transportation for up to 7 days.
- During transportation, avoid frequent freeze-thaw cycles.

3.5 Sample safety

All samples are regarded potentially infectious. All samples should be extracted after being inactivated according to relevant national regulations.

Chapter 4 Operation

4.1 Preparing materials

Prepare the following materials:

Table 3 User-supplied materials

Type	Item	Description
Equipment	Grinder	Brand: ServiceBio Model: KZ- III -F
	Mini centrifuge	With a speed no less than 12000 rpm
	Vortex mixer	With a speed no less than 2500 rpm
	Thermomixer compact	It can be replaced by a water bath
	1.5 mL magnetic rack	None
Equipment	Pipette	1 mL/200 μL/20 μL/10 μL
Reagent	Absolute Ethanol	Analytically pure
	PBS buffer	None

Type	Item	Description
Consumables	Pipette tips	1 mL/200 µL/20 µL/10 µL
	Centrifuge tube	• 1.5 mL • DNase-free, RNase-free

4.2 Pretreating samples

Pretreat samples according to the sample type.

Tips
- For mycoplasma, chlamydia, and viral samples, no need to grind them. Take out a new 1.5 mL centrifuge tube, and add 200 µL of sample, 400 µL of Buffer PML and 40 µL of Proteinase K into the tube. Mix the tube thoroughly by vortexing and go to the next step.
- Before use, frozen samples need must be thawed and mixed thoroughly.
- If the sample volume is less than 200 µL, use PBS buffer to bring the total volume to 200 µL.

Perform the following steps:

1. Take a new grinding tube from the kit.
2. Add an appropriate amount of sample and reagent to the tube according to the different sample types.
   - Blood sample
     - For the whole blood samples, add 50 µL to 100 µL of sample (manual extraction) or 50 µL to 80 µL of sample (extraction on MGISP-NE32RS) into the tube. Add PBS buffer to bring the total volume to 200 µL.
     - For the serum or plasma samples, add 200 µL of sample into the tube.
   - Sterile body fluids
     - For the sputum samples, add 10 µL to 50 µL of sample into the tube. Add PBS buffer to bring the total volume to 200 µL.
     - For the urine, cerebrospinal fluid, or bronchoalveolar lavage fluid samples, add 200 µL of sample into the tube.
   - Swab sample
     - For dry swab samples, add an appropriate volume of PBS buffer to immerse the swab, vortex to mix thoroughly, and then take 200 µL of the supernatant and add it into the grinding tube.
     - For swab samples containing preservative solution, vortex to mix the sample thoroughly. Aspirate 200 µL of the supernatant and add it into the tube.

- Stool sample
  - For the fresh or frozen stool samples, add 2 mg to 10 mg of sample (manual extraction) or 1 mg to 4 mg of sample (extraction on MGISP-NE32RS), and 200 µL of PBS buffer into the tube.
  - For the stool samples with stool preservative (Sample: Stool preservative=1:4), add 8 µL to 40 µL of sample (manual extraction) or 4 µL to 16 µL of sample (extraction on MGISP-NE32RS) and PBS buffer to bring the total volume to 200 µL.
- Tissue sample

  Perform the following steps:

  a. Prepare 5 mg to 50 mg of sample (manual extraction) or 5 mg to 20 mg of sample (extraction on MGISP-NE32RS), and cut sample into sesame-sized pieces with a scalpel.

  b. Add prepared sample and 200 µL of PBS buffer into the tube.

  c. Place the grinding tube in the grinder (Brand: ServiceBio, Model: KZ-III-F), and set the running time to 45 seconds, pause time to 15 seconds, frequency to 60 Hz, cycle number to 2 and temperature to 4 ℃ .

3. Add 400 µL of Buffer PML and 40 µL of Proteinase K into the tube. Tighten the cap and vortex to mix it.
4. Place the grinding tube in a vortex mixer, set the maximum speed (approximately 2000 rpm), and vortex it for 10 minutes.
5. (Optional) For viral nucleic acid extraction, place the centrifuge tube in a thermomixer, set the speed to 1000 rpm to 1500 rpm, and incubate at 95 ℃ for 10 minutes.
6. Use a pipette to transfer the liquid from the tube to a new 1.5 mL centrifuge tube, and label it until use.

4.3 Extracting the nucleic acids

Tips
- Before extraction, add absolute ethanol into Buffer WB I and Buffer WB II according to the label, and mix them before use.
- You can extract the nucleic acids manually or on automation devices. For automated nucleic acid extraction, ensure that you prepare applicable consumables.

4.3.1 Extracting the nucleic acids manually

Tips
- Before use, take out the magnetic beads 30 minutes in advance and place the sample at room temperature until its temperature is consistent with the room temperature.
- After mixing, briefly centrifuge the tube if beads remain on the tube wall or cap.

Perform the following steps:

1. Take out the pretreated sample tube, add 400 µL of absolute ethanol and 60 µL of magnetic beads into the tube, and mix the tube thoroughly by vortexing. Set it aside for 10 minutes, during which vortex the tube for 10 seconds every 2 minutes.
2. Place the tube on the magnetic rack for 2 minutes. When Magnetic Beads T is adsorbed completely on the tube wall, use a pipette to carefully remove the liquid.
3. Remove the tube from the rack, add 900 µL of Buffer WBI into the centrifuge tube, and mix it thoroughly by vortexing 2 minutes. Place the tube on the magnetic rack for 2 minutes. When Magnetic Beads T is adsorbed completely on the tube wall, use a pipette to carefully remove the liquid.

   Tips After adding Buffer WBI, mix the tube thoroughly by vortexing. Otherwise, the purity of the nucleic acid product may be affected.
4. Repeat step 3. Remove the residual liquid in the tube as much as possible.
5. Remove the tube from the rack, add 900 µL of Buffer WBII into the centrifuge tube, and mix it thoroughly by vortexing 2 minutes. Place the tube on the magnetic rack for 2 minutes. When Magnetic Beads T is adsorbed completely on the tube wall, use a pipette to carefully remove the liquid.
6. Repeat step 5. Remove the residual liquid in the tube as much as possible.
7. Place the tube on the magnetic rack. Decap and dry the tube for 5 to 10 minutes until no obvious reflection appears on the surface of the magnetic beads.

   Tips When the tube is being air-dried, ensure that the ethanol evaporates completely, but do not over-dry to avoid affecting the elution of the nucleic acid product.
8. Remove the tube from the rack, add 50 µL to 150 µL of elution buffer into the centrifuge tube, and mix it thoroughly by vortexing. Place the tube at a thermomixer compact at 1000 rpm to 1500 rpm for 5 minutes.

   Tips For nucleic acid extraction from viral samples, the elution temperature of the thermomixer compact should be set to 65 ℃ .

9. Place the tube on the magnetic rack for 2 minutes. When Magnetic Beads T is adsorbed completely, carefully transfer the nucleic acid solution into a new 1.5 mL centrifuge tube, label it until use.

   The extracted product can be directly used for downstream experiments. If it is not used immediately, store them at -80 ℃ and below for a long time.

   Tips If there are residual magnetic beads in the nucleic acid solution, you can extend the magnetic separation time appropriately to avoid affecting subsequent experiments.

4.3.2 Extracting the nucleic acids automatically on MGISP-NE32RS

4.3.2.1 Preparing consumables

According to the following table, prepare consumables for a workflow of automated extraction on MGISP-NE32RS and place them at room temperature until use:

Table 4 Required automated consumables for extraction on MGISP-NE32RS

Name	Brand	Cat. No.	Quantity
2.2 mL 96-well V-bottom deep-well plate (MGISP-NE32)	MGI	091-000444-00	2
Plastic Magnetic Wand Cover	MGI	1000022599	2

4.3.2.2 Preparing samples

You can extract 1 to 32 samples on MGISP-NE32RS. The MGISP-NE32RS supports extraction experiments for 32 samples, with each 6 columns completing the extraction of 8 samples per run.

Ensure that samples to be extracted have been pretreated.

4.3.2.3 Preparing reagents

Perform the following steps:

1. Add absolute ethanol into the Buffer WB I according to the label, and mark it on the label. Mix the tube and store it at room temperature until use.
2. Add absolute ethanol into the Buffer WB II according to the label, and mark it on the label. Mix the tube and store it at room temperature until use.

3. Take out one 2.2 mL 96-well V-bottom deep-well plate (1 to 16 samples) or two 2.2 mL 96-well V-bottom deep-well plate (17 to 32 samples), and add reagents into the plate(s). Ensure that no bubbles exist at the bottom of the deep-well plate and no liquid remains on the side walls.

Table 5 Required reagent volume for each well

Reagent name	Col. No.	Adding volume/well
Pretreated sample (Sample+Buffer PML +Proteinase K)	1 and 7	Refer to *Extracting the nucleic acids automatically on MGISP-NE32RS on Page 8*
Absolute ethanol		400 µL
Magnetic Beads T		60 µL
Buffer WB I	2 and 8	900 µL
Buffer WB I	3 and 9	900 µL
Buffer WB II	4 and 10	900 µL
Elution buffer	5 and 11	50 µL to 150 µL
Buffer WB II	6 and 12	900 µL

4.3.2.4 Starting extraction

Perform the following steps:

1. Power on MGISP-NE32RS, and the device starts self-test.

   Self-test takes approximately 10 seconds.

   CAUTION If the device shows abnormal behavior or displays errors after startup, or if there are fault alarms and prompts during self-test, immediately turn off the power and contact the technical support.

2. Configure a script by using either of the following methods:

   - In the main interface, tap **New** to enter the file editing interface. Edit the script according to step 5 below for different samples.
   - Insert the USB flash drive, and tap **Run** in the main interface. Select the script file from the USB flash drive, tap **Import** in the lower left corner, and you will be prompted that the script is successfully imported.

3. Tap **Device**, and select a script according to the sample type.

   - For the microbial culture medium sample, select **JB_010_V1**.
   - For the viral sample, select **JB_010_V_V1**.

4. Place the pre-filled plate(s) into the position, and install the plastic magnetic wand cover.

5. Tap **Run**. The device starts extraction according to the following table. The whole workflow takes about 33 min.

   During the workflow, tap **Stop** to stop the workflow, **Pause** to pause the workflow, and tap **Pause/Reset** to suspend the workflow and raise the magnetic rods if required.

   The temperature setting for different samples is shown as below:

   - Microbial culture medium sample

     Lysis Temp: OFF

     Elution Temp: OFF

   - Viral sample:

     Lysis Temp: 95 ℃ . The lysis heating stops at step 3.

     Elution Temp: 65 ℃ . The elution heating starts at step 10.

Table 6 Automated extraction settings

Step No.	Col. No.	Name	Wait time (min:ss)	Mix time (min:ss)	Mag time (min:ss)	Volume (μL)	Mixing method	Collect method
1	1	Bind	00:00	08:00	02:00	1000	Fast	Strong
2	1	Bind	00:00	00:00	00:10 x 3	1000	Slow	Cycle
3	2	Wash I	00:00	01:00	01:00	900	Fast	Strong
4	2	Wash I	00:00	00:00	00:10 x 3	900	Slow	Cycle
5	3	Wash I	00:00	01:00	01:00	900	Fast	Strong
6	3	Wash I	00:00	00:00	00:10 x 3	900	Slow	Cycle
7	4	Wash II	00:00	01:00	01:00	900	Fast	Strong
8	4	Wash II	00:00	00:00	00:10 x 3	900	Slow	Cycle
9	6	Wash II	00:00	01:00	01:00	900	Fast	Strong
10	6	Wash II	00:00	00:00	00:10 x 3	900	Slow	Cycle
11	5	Elute	04:00	03:30	02:00	100	Fast	Strong
12	5	Elute	00:00	00:00	00:10 x 3	100	Slow	Cycle
13	2	Beads	00:00	00:00	00:10	900	Fast	Normal

⚠ CAUTION After the workflow is completed, wait for the completion alert sound to finish and ensure that the robotic arm is no longer moving before opening the compartment door. Otherwise, the device may become jammed.

6. After the program ends, remove and transfer the plastic magnetic wand cover to the medical waste bag.

7. Take out the plate(s) immediately, transfer the extracted nucleic acid from columns 5 and 11 into new 8-strip tubes, and securely cap the tubes. The extracted product can be used directly for subsequent experiments or stored at -80 ℃ until use.

   ⚠ CAUTION After the workflow is completed, immediately take out the deep-well plates. Do not leave the product in the temperature control position for an extended period, as this may affect the product quality.

8. Dispose of the used deep-well plate in the designated waste area. Use lint-free paper moistened with 75% alcohol to wipe the interior surfaces of the compartment, and then close the compartment door. In the main interface, tap **UV Lamp**, and set the time to 30 minutes. Tap **Confirm** to turn on the UV lamp.

Chapter 5 Warnings and precautions

- This product is for research use only. Please read the instructions for use carefully before use.
- Before experiment, be sure to be familiar with and master the operation methods and precautions of various devices to be used.
- Direct contact with skin and eyes should be avoided for all samples and reagents. Do not swallow. If accidental ingestion occurs, please get medical attention immediately. If skin exposure occurs, rinse with large amounts of water and get medical attention if irritation persists.
- Do not freeze and thaw frozen samples frequently. Otherwise, the quality of the nucleic acid may decrease.
- Before use, take out all components in the reagent kit, equilibrate to room temperature (10 ℃ to 30 ℃) and mix them thoroughly before adding to wells. Unless otherwise specified, operate at room temperature.
- After the experiment, ensure that the reagent bottle caps are tightly closed, especially for Buffer WB1 and Buffer WBII with absolute ethanol.
- All samples and wastes should be disposed of in accordance with relevant regulations.
- Do not use expired products.

Appendix 1 Manufacturer information

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