Part No.: H-020-000720-00

MGIEasy

Total RNA Extraction Set

Instructions for Use

Version: 3.0

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 Total RNA Extraction Set. The version of the instructions for use is 3.0 and the set version is 1.0.

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Revision history

Version	Date	Description
3.0	December 25, 2023	Updated the operation
2.0	October 27, 2023	Updated instructions and tips for 4.1, 4.2 and 4.3
1.0	May 15, 2023	Initial release

Contents

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Chapter 1 Introduction

1.1 Product name

MGIEasy Total RNA Extraction Set

1.2 Specifications

Set name	Model	Component	Cat. No.	Specification
MGIEasy Total RNA Extraction Set Cat. No.: 940-000880-00	MRT96	MGIEasy Total RNA Extraction Kit	940-000877-00	96 RXN/Kit
		DNase I	940-000879-00	
MGIEasy Total RNA Extraction Set Cat. No.: 940-000875-00	MRT384	MGIEasy Total RNA Extraction Kit	940-000878-00	384 RXN/Kit
		DNase I	940-000876-00	

1.3 Intended use

This set is used to extract high-quality and high-purity total RNA from cell, animal tissue (fresh or frozen), blood and other samples.

1.4 Working principle

By using this product, salt ions with high concentration lyse and release RNA from the animal cell, animal tissue (fresh or frozen at -80 ℃) and blood samples. The released RNA is then captured by magnetic beads and washed by specific wash buffer to remove proteins, salt and other impurities. After being dried, the RNA in magnetic beads is eluted by elution buffer and high-purity total RNA is obtained.

1.5 Main components

Tips To avoid frequent freeze-thaw cycles, Buffer RDD can be stored at room temperature not exceeding 30 ℃ .

Table 1 MGIEasy Total RNA Extraction Set (MRT96) Cat. No.: 940-000880-00

Kit name	Component	Specification	Storage condition	Validity period	Transportation condition
MGIEasy Total RNA Extraction Kit Cat. No.: 940-000877-00	Buffer LY	29 mL/tube×1	2 ℃ to 30 ℃	12 months	2 ℃ to 30 ℃
	Buffer WB I	81 mL/tube×1			
	Buffer WB II	27 mL/tube×1			
	RNase Free Water	15 mL/tube×1			
	Proteinase K	2 mL/tube×1			
	Magnetic Beads T	6 mL/tube×1			
	Buffer LYR	168 mL/tube×2			
DNase I	DNase I	0.8 mL/tube×1	-25 ℃ to -15 ℃		-25 ℃ to -15 ℃
Cat. No.: 940-000879-00	Buffer RDD	15 mL/tube×1	-25 ℃ to 30 ℃		-25 ℃ to 30 ℃

Table 2 MGIEasy Total RNA Extraction Set (MRT384) Cat. No.: 940-000875-00

Kit name	Component	Specification	Storage condition	Validity period	Transportation condition
MGIEasy Total RNA Extraction Kit Cat. No.: 940-000878-00	Buffer LY	116 mL/tube×1	2 ℃ to 30 ℃	12 months	2 ℃ to 30 ℃
	Buffer WB I	323 mL/tube×1			
	Buffer WB II	108 mL/tube×1			
	RNase Free Water	60 mL/tube×1			
	Proteinase K	8 mL/tube×1			
	Magnetic Beads T	24 mL/tube×1			
	Buffer LYR	672 mL/tube×2			
DNase I	DNase I	0.8 mL/tube× 4	-25 ℃ to -15 ℃		-25 ℃ to -15 ℃
Cat. No.: 940-000876-00	Buffer RDD	61 mL/tube× 1	-25 ℃ to 30 ℃		-25 ℃ to 30 ℃

Chapter 2 Applicable device

- MGISP-960RS High-throughput Automated Sample Preparation System
- MGISP-NE384RS Automated Nucleic Acid Extractor

Chapter 3 Sample requirements

3.1 Applicable sample

This product is applicable to samples from cultured eukaryotic cell, solid tissue and blood of human or animals, and prokaryotic cell such as G+ bacteria and G- bacteria.

3.2 Sample amount requirements

		Manual extraction	MGISP-960RS	MGISP-NE384RS
Human whole blood		100 μL to 200 μL	100 μL to 200 μL	100 μL to 200 μL
Animal tissue	Tissue of liver, spleen and kidney	1 mg to 20 mg	1 mg to 30 mg	5 mg to 30 mg
	Tissue of heart and lungs	5 mg to 15 mg	2 mg to 5 mg	5 mg to 20 mg
Cell		1×10^5 to 5×10^6	1×10^5 to 2.5×10^6	1×10^5 to 1×10^6
Bacteria		5×10^7 to 5×10^9	5×10^7 to 5×10^9	5×10^7 to 5×10^9
Yeast		5×10^7	5×10^7	5×10^7

Tips The amount of human whole blood sample should be adjusted according to the white blood cells count in the blood.

3.3 Sample storage

- For human whole blood samples, it is recommended to use EDTA-anticoagulated whole blood or sodium citrate-anticoagulated whole blood.
- For tissue samples, it is recommended to use fresh tissue or frozen tissue at -80 ℃ for up to 3 months.

- For cell or bacteria samples, it is recommended to use fresh precipitate cell or bacteria without culture medium or use frozen cell or bacteria at -80 ℃ for up to 6 months.
- Do not freeze and thaw frozen samples frequently. Otherwise, the RNA quality may decrease.
- Please thaw and mix the frozen samples thoroughly before use.
- Before use, take out all components in the reagent set, equilibrate to room temperature (10 ℃ to 30 ℃) and mix them thoroughly before adding to wells. If solid object appears, heat the reagent at 50 ℃ to redissovle it, which does not affect the reagent's extraction effect.

3.4 Sample transportation

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	Mini centrifuge	With a speed no less than 10000 rpm
	Plate centrifuge	None
	Tubular centrifuge	None
	Vortex mixer	None
	Thermomixer compact	It can be replaced by a water bath
	1.5 mL magnetic rack	None

Type	Item	Description
Equipment	Pipette	1 mL/200 μL/20 μL
	Grinding media	3 mm, zirconia, RNase-free
	Grinding mill	-10 ℃ , low-temperature
Reagent	Absolute ethanol	Analytically pure
	Lysozyme	Recommended brand: TIANGEN
	PBS solution	Recommended brand: Sangon Biotech
	DEPC	Recommended brand: Sangon Biotech
	1xTE Buffer	PH 8.0. Recommended brand: Sangon Biotech
	TritonX-100	Recommended brand: Aladdin
	Lysostaphin	Recommended brand: Coolaber
	β-Mercaptoethanol	Recommended brand: Aladdin
Consumables	Pipette tips	None
	Centrifuge tube	• 50 mL/1.5 mL/0.5 mL • DNase-free, RNase-free
	Tips	1 mL/200 μL/20 μL
	Glass beads, acid-washed	Recommended brand: Magen

4.2 Pretreating samples

It is necessary to pretreat samples before nucleic acid extraction.

4.2.1 Human whole blood

Tips The human whole blood here represents the EDTA-anticoagulated whole blood or sodium citrate-anticoagulated whole blood. It is recommended to extract nucleic acids directly from the human whole blood. If you need to store the human whole blood for a long time, pretreat it whose volume should be more than 200 μL, and store it at -80 ℃ for up to 1 month. Blood is complicated in structure, so during blood pretreatment, RNA from blood sample may degrade, but the purity is not affected.

Perform the following steps:

1. Use a new centrifuge tube. Add 200 µL to 1000 µL of fresh human whole blood and 1× Buffer LYR into the tube. The adding volume of 1× Buffer LYR is 5 times that of fresh human whole blood.

   Tips
   - To mix thoroughly, the volume of the mixture of blood and 1× Buffer LYR cannot exceed three-fourths of the tube height.
   - For poultry blood, you can determine the required amount according to your needs and directly complete human whole blood lysis.

2. Incubate the tube on ice for 10 to 15 minutes, during which vortex the tube twice with 5 seconds for each time.

   Tips
   - During incubation, when the mixture is translucent, the red blood cell is lysed.
   - The incubation time could be extended to 20 minutes if necessary.

3. Place the tube into a centrifuge with a speed of 2100 rpm (about 400 ×g), centrifuge it at 4 ℃ for 10 minutes, and remove the supernatant completely.

   Tips
   - After centrifuge, white blood cells may become pellets. Please ensure to remove the supernatant completely.
   - Trace red blood cells will make pellets formed by white blood cells become red. But the color will fade after wash.

4. Add 1× Buffer LYR whose volume is twice that of human whole blood into the centrifuge tube containing white blood cell pellet and resuspend the cell.
5. Place the tube into a centrifuge with a speed of 2100 rpm (about 400 ×g), centrifuge it at 4 ℃ for 10 minutes, and remove the supernatant completely.

4.2.2 Animal tissue

Perform the following steps:

1. Use a new 1.5 mL centrifuge tube. Add 1 mg to 20 mg of fresh or frozen tissue at -80 ℃ into the tube.
2. Add 100 µL to 500 µL of Buffer LY and 2 to 5 RNase-free zirconia beads into the tube. Place the tube into an electronic homogenizer and start reaction with a frequency of 70 Hz at 4 ℃ for 1 minute.

   Tips After the sample is grinded by the Buffer LY, a large amount of bubbles will appear. Therefore, it is recommended to reserve partial absolute ethanol during lysis buffer preparation. Add the reserved absolute ethanol into the centrifuge tube containing pretreated samples to remove bubbles and transfer the samples into the plate for sample according to the requirement of total volume.

3. Aspirate the supernatant slowly for extraction.

4.2.3 Cell

Tips The following methods are applicable to extract 10 μg to 30 μg of total RNA from 1x10^6 cultured eukaryotic cells.

4.2.3.1 Cell suspension for collection

Perform the following steps:

1. Add cells to a new 1.5 mL centrifuge tube and estimate the cell count.
2. Collect the cells to the tube and centrifuge it in a centrifuge at 300 ×g for 5 minutes.
3. Remove the supernatant of culture medium.

4.2.3.2 Trypsin treatment

Perform the following steps:

1. Add the cells to a new 1.5 mL centrifuge tube, estimate the cell count, and remove the culture medium.
2. Add the PBS solution to wash cells and remove the PBS solution.
3. Add the PBS solution containing 0.1% to 0.25% trypsin into the tube.
4. When the cells detach from the wall of the tube, add the culture medium with serum to inactivate trypsin. Transfer the cell solution into a RNase-free centrifuge tube and centrifuge it in a centrifuge at 300 ×g for 5 minutes.
5. Collect cell pellets and remove the supernatant.

   Tips When collecting cells, completely remove the cell culture medium. Otherwise, the cell may not be lysed completely, which affects the combination between RNA and magnetic beads, and even the RNA yield.

4.2.4 Bacteria

Perform the following steps:

1. Use a new 1.5 mL centrifuge tube. Estimate the bacteria number and collect the bacteria to the tube and centrifuge it in a centrifuge at 500 ×g for 5 minutes.
2. Remove the supernatant of culture medium.
3. Add 100 μL of lysozyme solution into the tube. The concentration and dissolution method are as follows:

Bacteria type	Concentration of lysozyme	Dissolution method
G- bacteria	1 mg/mL	1xTE Buffer
Most G+ bacteria	15 mg/mL	1xTE Buffer with 1.2% TritonX-100

Tips
- Most G+ bacteria here represents clostridium butyricum, clostridia spotogenes and listeria monocytogenes.
- For the extraction from staphylococcus aureus and staphylococcus epidermidis, mix 50 mM Tris-HCl (pH 7.5) and lysostaphin to prepare lysostaphin solution (2.4 U/μL). Store the solution at -20 ℃ and avoid frequent freeze-thaw cycle.

4. Vortex the tube to mix it thoroughly. Place the G- bacteria and most G+ bacteria at room temperature for 3 to 5 minutes and 5 to 10 minutes, respectively.

   Tips For staphylococcus aureus and staphylococcus epidermidis, add 10 μL of lysostaphin solution and 50 μL to 90 μL of PBS solution into the tube, vortex the tube for 10 seconds and incubate it for 30 minutes. At this time, extract the nucleic acids from the sample directly.

5. Add 300 μL of Buffer LY and 20 μL of Proteinase K into the tube. Mix the tube thoroughly and let stand for 5 minutes.
6. Add 60 μL of Magnetic Beads T and 300 μL of absolute ethanol into the tube. Mix the tube thoroughly and let stand for 8 minutes.

4.2.5 Yeast

Tips It is recommended that the number of yeasts should not exceed $5x10^7$.

Perform the following steps:

1. Estimate the count of the bacteria, add the bacteria to a new 1.5 mL centrifuge tube and centrifuge the tube in a centrifuge at 500 ×g for 5 minutes.
2. Slowly remove the culture medium.
3. Add the acid-washed glass beads into the tube until the volume reaches 100 μL mark. Then, add 300 μL of Buffer LY into the tube.
4. Place the tube into an electronic homogenizer and start reaction with a frequency of 70 Hz at 0 ℃ for 1 minute. Grind the sample again after 30 seconds.

   Tips A large amount of bubbles may appear in the tube after grinding. Just add 300 μL of absolute ethanol to remove the bubbles.

5. Aspirate the supernatant for extraction. Do not touch the glass beads in the process.

4.3 Extracting the nucleic acids

Tips
- You can extract the nucleic acids manually or on automation devices. For automated nucleic acid extraction, ensure that you prepare applicable consumables.
- During extraction on MGISP-960RS or MGISP-NE384RS, pretreat cell, bacteria, human whole blood and other samples, according to the requirement of 50 μL/well, use an appropriate volume of 1× PBS solution to disperse samples evenly, and use pipette to transfer samples into the 96-well plate for the lysis buffer.

4.3.1 Extracting the nucleic acids manually

Perform the following steps:

1. Prepare the lysis buffer according to the sample type:

Table 4 Proportion for lysis buffer

Sample type	Human whole blood	Animal tissue	Cell	Bacteria	Yeast
Magnetic Beads T	60 μL	60 μL	60 μL	60 μL	60 μL
Proteinase K	20 μL	20 μL	20 μL	20 μL	20 μL
Buffer LY	300 μL	300 μL	300 μL	300 μL	300 μL
Absolute ethanol	400 μL	300 μL	300 μL	300 μL	300 μL
β-Mercaptoethanol	6 μL	/	/	/	/

Tips
- The prepared lysis buffer is required to be used within 30 minutes. If you want to prepare the buffer in advance, add Proteinase K before adding the buffer to the plate to avoid inactivating Proteinase K caused by long-time preparation.
- During extraction from the human whole blood, add β-Mercaptoethanol, whose volume is 2% that of Buffer LY, to the tube containing Buffer LY. For example, for 1 mL of Buffer LY, you need to add 20 μL of β-Mercaptoethanol. Use the reagent immediately after preparation. The prepared reagent can be stored at 4 ℃ for 1 month. If solid object appears, heat the reagent at 37 ℃ to redissovle it.

2. Add the lysis buffer into the centrifuge tube with pretreated samples. Vortex the tube to mix thoroughly and place at room temperature for 8 minutes during which vortex the tube 2 to 3 times with 5 seconds for each time.

3. Place the tube on the magnetic rack and let stand for 2 to 3 minutes during which slowly invert the tube on the magnetic rack to wash the Magnetic Beads T on the tube wall and cap. When Magnetic Beads T is adsorbed completely, use a pipette to remove the supernatant.
4. Add 700 µL of Buffer WB I to the tube. Vortex the tube for 1 minute to mix thoroughly and place the tube on the magnetic rack for 1 minute during which slowly invert the tube on the magnetic rack. When Magnetic Beads T is adsorbed completely, use a pipette to remove the supernatant.
5. (Optional) For extraction from the human whole blood, repeat step 4 once. Add 700 µL of Buffer WB II to the tube. Vortex the tube for 1 minute to mix thoroughly, briefly centrifuge and place the tube on the magnetic rack for 1 minute. When Magnetic Beads T is adsorbed completely, use a pipette to remove the supernatant.
6. Decap and dry the tube for 5 to 10 minutes.
7. Add 8 µL of DNase I and 72 µL of Buffer RDD into the tube. Vortex the tube for 1 minute to mix thoroughly and place the tube at room temperature for 15 minutes during which vortex the tube for 10 seconds every 5 minutes.
8. Add 700 µL of Buffer WB I or Buffer WB II (for human whole blood extraction only) into the tube. Vortex the tube for 1 minute to mix thoroughly and let stand at room temperature for 3 minutes. Centrifuge the tube briefly and place on magnetic rack for 1 minute during which slowly invert the tube on the magnetic rack. When Magnetic Beads T is adsorbed completely, use a pipette to remove the supernatant.
9. Add 700 µL of Buffer WB II to the tube. Vortex the tube for 1 minute to mix thoroughly, centrifuge briefly and place the tube on the magnetic rack for 1 minute. When Magnetic Beads T is adsorbed completely, use a pipette to remove the supernatant.
10. Repeat step 9 once.

    Tips Skip this step for human whole blood extraction.
11. Decap and dry the tube for 5 to 10 minutes.
12. Add 80 µL of RNase Free Water to the tube. Vortex the tube for 1 minute and let stand at room temperature for 5 minutes. Centrifuge briefly and place the tube on the magnetic rack. When Magnetic Beads T is adsorbed completely, aspirate the supernatant. The aspirated supernatant is the required product. Place the product at -80 ℃ for storage.

4.3.2 Extracting the nucleic acids automatically on MGISP-960RS

4.3.2.1 Preparing consumables

According to the following table, prepare consumables for a workflow of automated extraction on MGISP-960RS and place them at room temperature until use:

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	7
2.2 mL V-bottom 96-well deep-well plate	MGI	1000008088	4 or 6 (for human whole blood extraction only)
1.3 mL U-bottom 96-well deep-well plate	MGI	1000004644	3 or 2 (for human whole blood extraction only)
Hard-shell thin-wall 96-well skirted PCR plates	MGI	1000012059	1

4.3.2.2 Preparing samples

You can extract 1 to 96 samples on MGISP-960RS.

Perform the following steps:

1. Ensure that samples to be extracted have been pretreated.
2. Add samples to the 96-well deep-well plate with the volume no more than 50 µL for tissue, cell, bacteria and yeast samples or 200 µL for human whole blood extraction for each well, and pipette the samples 2 to 3 times. Ensure that no liquid exists on the wall of the well.

   Tips When the sample volume is greater than recommended, magnetic beads will be clustered, for which a part of beads is aspirated during liquid transfer and loss is caused. For example, when the input of liver cell ranges between 10 mg and 30 mg, magnetic beads may be clustered. The reduction of beads affects the yield but it does not affect the purity.

3. Place the 96-well deep-well plate with samples on ice until use.

4.3.2.3 Preparing reagents

Perform the following steps:

1. Add absolute ethanol into the Buffer WB I according to the label.
2. Add absolute ethanol into the Buffer WB II according to the label.

3. Prepare the lysis buffer according to the sample type:

Table 5 Proportion for lysis buffer

Sample type	Human whole blood	Animal tissue	Cell	Bacteria	Yeast
Magnetic Beads T	60 µL	60 µL	60 µL	60 µL	60 µL
Proteinase K	20 µL	20 µL	20 µL	20 µL	20 µL
Buffer LY	300 µL	300 µL	300 µL	300 µL	300 µL
Absolute ethanol	400 µL	300 µL	300 µL	300 µL	300 µL
β-Mercaptoethanol	6 µL	/	/	/	/

Tips

- The prepared lysis buffer is required to be used within 30 minutes. If you want to prepare the buffer in advance, add Proteinase K before adding the buffer to the plate to avoid inactivating Proteinase K caused by long-time preparation.
- During extraction from the human whole blood, add β-Mercaptoethanol, whose volume is 2% that of Buffer LY, to the tube containing Buffer LY. For example, for 1 mL of Buffer LY, you need to add 20 µL of β-Mercaptoethanol. Use the reagent immediately after preparation. The prepared reagent can be stored at 4 ℃ for 1 month. If solid object appears, heat the reagent at 37 ℃ to redissovle it.

4. Prepare three or two (only for human whole blood extraction) 1.3 mL U-bottom 96-well deep-well plates, three or six (only for whole blood extraction) 2.2 mL V-bottom 96-well deep-well plates and a hard-shell thin-wall 96-well skirted PCR plate. Add reagents according to the following table:

Reagent name	Adding volume	Plate type
RNase Free Water	100 µL/well	1.3 mL U-bottom 96-well deep-well plate
Buffer WB I	1500 µL/well	2.2 mL V-bottom 96-well deep-well plate
Buffer WB II	1500 µL/well	2.2 mL V-bottom 96-well deep-well plate
Buffer WB II (only for human whole blood extraction)	800 µL/well	2.2 mL V-bottom 96-well deep-well plate

Reagent name	Adding volume	Plate type
Lysis buffer	680 µL/well or 786 µL/well (only for human whole blood extraction)	1.3 mL U-bottom 96-well deep-well plate or 2.2 mL V-bottom 96-well deep-well plate (only for human whole blood extraction)
DNase I + Buffer RDD	8 µL/well + 72 µL/well	1.3 mL U-bottom 96-well deep-well plate
Waste plate	/	2.2 mL V-bottom 96-well deep-well plate
Waste plate	/	2.2 mL V-bottom 96-well deep-well plate
Product plate	/	Hard-shell thin-wall 96-well skirted PCR plates

4.3.2.4 Starting extraction

Perform the following steps:

1. Switch to the | position to power on the device.
2. Turn on the computer and the desktop appears. Double-click  to run the software.
3. Select **User** and **Real**. Enter the password.
4. Click **Login** to enter the main interface.
5. On the upper-right corner of the control software, click  and select **WDesigner**. The home interface is displayed.
6. Ensure that the application file in the *.wfex* format has been prepared.
7. Click  in the toolbar and find the file location in the pop-up window.
8. Select the file and click **Open**, fill in the **Application** and **Project**, and click **Confirm** to save the application file. Then this application file can be executed in the control software.
9. After the file is imported successfully, click  in the toolbar.
10. Click **Initialize** on the top of the interface to start initializing.

    You will be prompted after a successful initialization.

11. Click ☰ on the left of the interface, and select **Clean**>**Pre-clean**>**Start**.
12. Follow the on-screen instructions to complete operations and click **Continue**. The UV lamp and air filter start working.

    ⚠ CAUTION Ultraviolet radiation is harmful to the human body, so do not open the door after the pre-clean starts.

13. Import the application scripts according to *MGISP-100&MGISP-960 Application Script Installation Instructions*.
14. Tap ☰ >**Run Wizard** to enter the Run Wizard interface.
15. Click the drop-down list of **Solution** and select **JB-A09-137 MGIEasy Total RNA Extraction Kit_RV1.0_SV1.0**. Click the drop-down list of **Script**, select **JB-A09-137 MGIEasy Total RNA Extraction Kit_RV1.0_SV1.0.py** for cell, tissue, bacteria and yeast samples, and select **JB-A09-137 MGIEasy Total RNA Extraction Kit (Blood)_RV1.0_SV1.0.py** for the human whole blood sample. Place samples, reagents and consumables according to the following table and figure:

Name	Position
250 μL automated filter tips	Pos1 to Pos7
RNase Free Water	Pos13
Buffer WB I	Pos14
Buffer WB II	Pos15
Buffer WB II (only for human whole blood)	Pos23
Lysis buffer	Pos20
DNase I + Buffer RDD	Pos21
Waste plate	Pos16
Waste plate	Pos18
RNA product (empty PCR plate)	Pos12

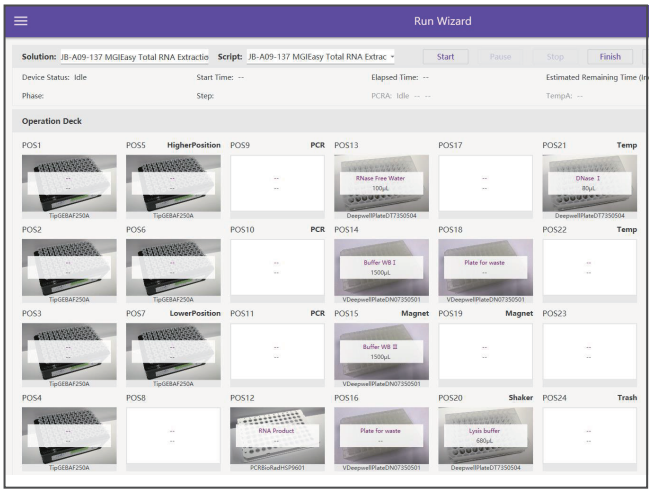

Figure 1 Plate map for tissue, cell, bacteria and yeast samples

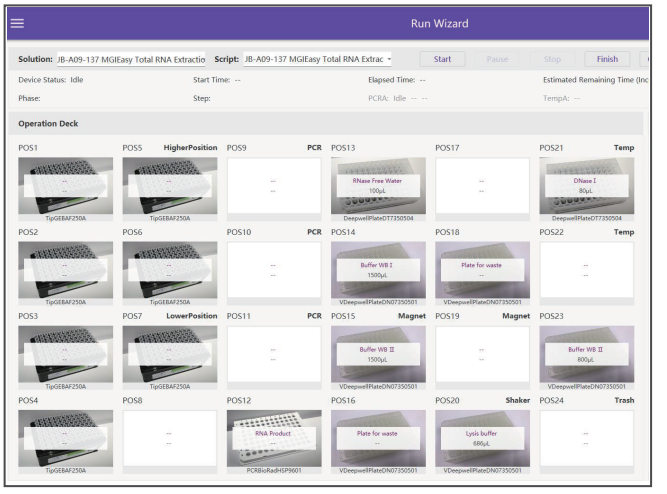

Figure 2 Plate map for human whole blood sample

16. Click **Start**. The extraction workflow starts. It takes roughly 1.5 hours.

    During the workflow, click **Pause** to pause and click **Resume** to resume the workflow if required.

17. At the end of the workflow, remove the RNA product from Pos12.

    If the product is not used immediately, seal and store it in a freezer at -80 ℃ .

18. Dispose of the used deep-well plates, PCR plates and waste bag.

    If no experiment is to be conducted on the rest of the day, clean the operation deck of the device according to *MGISP-100&MGISP-960 Cleaning Instructions*.

4.3.3 Extracting the nucleic acids automatically on MGISP-NE384RS

4.3.3.1 Preparing consumables

According to the following table, prepare consumables for a workflow of automated extraction on MGISP-NE384RS and place them at room temperature until use:

Name	Brand	Cat. No.	Quantity
2.2 mL V-bottom 96-well deep-well plate	MGI	1000008088	96 preps: 6 or 7 (only for human whole blood)
			384 preps: 24 or 28 (only for human whole blood)
96-well tips comb	MGI	1000025661	1 (96 preps)
			4 (384 preps)

4.3.3.2 Preparing samples

You can extract 96 to 384 samples on MGISP-NE384RS.

Perform the following steps:

1. Ensure that samples to be extracted have been pretreated.
2. Add samples to the 96-well deep-well plate. Seal the plate and vortex it until the magnetic beads T is mixed thoroughly. Remove the seal and centrifuge the plate to ensure that no liquid exists on the wall of the well.

Tips When the sample volume is greater than recommended, magnetic beads will be clustered, for which a part of beads is remained in the tips comb during liquid absorption and transfer. For example, when the input of liver cell ranges between 15 mg and 30 mg, magnetic beads may be clustered. The reduction of beads affects the yield but it does not affect the purity.

4.3.3.3 Preparing reagents

Perform the following steps:

1. Add absolute ethanol into the Buffer WB I according to the label.
2. Add absolute ethanol into the Buffer WB II according to the label.
3. Prepare the lysis buffer according to the sample type:

Table 6 Proportion for lysis buffer

Sample type	Human whole blood	Animal tissue	Cell	Bacteria	Yeast
Magnetic Beads T	60 µL	60 µL	60 µL	60 µL	60 µL
Proteinase K	20 µL	20 µL	20 µL	20 µL	20 µL
Buffer LY	300 µL	300 µL	300 µL	300 µL	300 µL
Absolute ethanol	400 µL	300 µL	300 µL	300 µL	300 µL
β-Mercaptoethanol	6 µL	/	/	/	/

Tips
- The prepared lysis buffer is required to be used within 30 minutes. If you want to prepare the buffer in advance, add Proteinase K before adding the buffer to the plate to avoid inactivating Proteinase K caused by long-time preparation.
- During extraction from the human whole blood, add β-Mercaptoethanol, whose volume is 2% that of Buffer LY, to the tube containing Buffer LY. For example, for 1 mL of Buffer LY, you need to add 20 μL of β-Mercaptoethanol. Use the reagent immediately after preparation. The prepared reagent can be stored at 4 ℃ for 1 month. If solid object appears, heat the reagent at 37 ℃ to redissovle it.

4. Take out six or seven (only for human whole blood) 2.2 mL V-bottom 96-well deep-well plates and add reagents according to the following table:

Reagent name	Adding volume
Lysis buffer and sample Tips For bacteria samples, you only need to add the pretreated samples.	680 μL/well or 786 μL/well (only for human whole blood)
Buffer WB I	700 μL/well
Buffer WB I (only for human whole blood)	700 μL/well
Buffer WB II	700 μL/well
Buffer WB II	700 μL/well
RNA product (containing RNase Free Water)	80 μL/well

4.3.3.4 Starting extraction

Perform the following steps:

1. Switch to the | position to power on the device.
2. Turn on the computer and the desktop appears. Double-click the icon of MGISP-NE384RS to run the software.
3. Select **User** and **Real**, and enter the password. Click **Login** to enter the main interface.
4. Click **Initialize** on the top of the interface to start initializing.

   You will be prompted after a successful initialization.
5. Click **Process manage** >  to import the script.
6. Click  > **Workflow**.

7. Select the script and place the sample, reagents and consumables according to different sample types.
   - For cell, tissue, bacteria and yeast samples, click the drop-down list of **Script**, select **MGIEasy Total RNA Extraction Kit_V1.0**, and place the sample, reagents and consumables according to the following table:

Reagent name	Position
Lysis buffer and sample Tips For bacteria samples, you only need to add the pretreated samples.	Pos1
Buffer WB I	Pos2
Buffer WB II	Pos4
Buffer WB II	Pos5
RNA product (with RNase Free Water)	Pos6

   - For the human whole blood sample, click the drop-down list of **Script**, select **MGIEasy Total RNA Extraction Kit** (**Blood**)_**V1.0** and place the sample, reagents and consumables according to the following table:

Reagent name	Position
Lysis buffer and sample Tips For bacteria samples, you only need to add the pretreated samples.	Pos1
Buffer WB I	Pos2
Buffer WB I (Used to replace the original one during human whole blood extraction)	Pos2
Buffer WB II	Pos3
Buffer WB II	Pos5
RNA product (with RNase Free Water)	Pos6

8. Click **Run**. The device starts extraction according to the following table.

   CAUTION Please add reagents manually according to the prompts within 15 minutes. Otherwise, RNA may degrade, which causes experimental failure.

   During the workflow, click **Pause** to pause and click **Resume** to resume the workflow if required.

   - Extraction from tissue, cell, bacteria and yeast samples

Before starting step 3, a message box appears to confirm that you have placed the deep-well plate with 8 µL of DNase I and 72 µL of Buffer RDD in each well into Pos3. Click **Confirm**. Step 3 starts.

Before starting step 4, a message box appears to confirm that you have added 700 µL of Buffer WB I into Pos3. Click **Confirm**. Step 4 starts.

Step No.	1	2	3	4	5	6	7	8
Step name	Lysis	Wash	Bind (manually)	Wash (manually)	Wash	Wash	Elution	Release
Position	1	2	3	3	4	5	6	2
Volume (µL)	680	700	80	780	700	700	80	700
Delay time (s)	0	0	300	20	0	0	300	0
Mix	True	True	True	True	True	True	True	True
Mix type	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mix rate	HighMiddle	HighMiddle	High	HighMiddle	HighMiddle	HighMiddle	High	High
Mix time (s)	400	60	600	180	60	60	180	10
Collect	True	True	False	True	True	True	True	False
Collect mode	Cycle	Cycle	/	Cycle	Cycle	Cycle	Cycle	/
Collect cycle	6	3	1	2	2	2	6	1
Collect time (s)	1	1	1	1	1	1	1	1
Dialog	False	True	True	False	False	False	False	True

- Extraction from human whole blood sample

  Before starting step 3, a message box appears to confirm that you have placed a new plate containing Buffer WB I into Pos2. Click **Confirm**. Step 3 starts.

  Before starting step 5, a message box appears to confirm that you have placed the deep-well plate with 8 µL of DNase I and 72 µL of Buffer RDD in each well into Pos4. Click **Confirm**. Step 5 starts.

  Before starting step 6, a message box appears to confirm that you have added 700 µL of Buffer WB II into Pos4. Click **Confirm**. Step 6 starts.

Step No.	1	2	3	4	5	6	7	8	9
Step name	Lysis	Wash	Wash	Wash	Bind	Wash	Wash	Elution	Release
Position	1	2	2	3	4	4	5	6	2
Volume (μL)	786	700	700	700	80	780	700	80	700
Delay time (s)	0	0	10	0	300	20	0	300	0
Mix	True	True	True	True	True	True	True	True	True
Mix type	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mix time (s)	480	60	60	60	600	180	60	180	10
Collect	True	True	True	True	False	True	True	True	False
Collect mode	Cycle	Cycle	Cycle	Cycle	/	Cycle	Cycle	Cycle	/
Collect cycle	6	3	3	2	/	2	2	6	/
Collect time (s)	1	1	1	1	1	1	1	1	1
Dialog	False	True	False	True	True	False	False	False	True

9. After the program ends, transfer the 96-well tips comb to the medical waste bag.
10. Remove the 96-well plate from Pos6 and transfer the product to a new tube.

    If the product is not used immediately, seal it and store it in a freezer at -80 ℃ .

Chapter 5 Warnings and precautions

- This product is for research use only. Please read the instructions for use of the product 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.
- All samples and wastes should be disposed of in accordance with relevant regulations.
- Do not use expired products.

Appendix 1 Manufacturer information

Manufacturer	Wuhan MGI Tech Co., Ltd.
Address	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R. China
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