Part No.: H-T-097

MGIEasy

Circulating DNA Extraction Max Kit (CDT-50)

Instructions for Use

Version: 4.0

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 Circulating DNA Extraction Max Kit (CDT-50). The version of the instructions for use is 4.0 and the set version is 1.0.

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Revision history

Version	Date	Description
4.0	November 11, 2024	1. Put urine extraction steps in tips in 4.3 2. Revised to “can process 1-24 samples” in 4.4.2 3. Revised reagents preparation for different number of samples in 4.4.3.2
3.0	July 30, 2024	1. Revised the kit components 2. Revised the operation of manual extraction 3. Added the sample type of serum 4. Added MGISP-NEX extraction 5. Deleted the specification of 25 preps
2.0	July 3, 2023	Updated the operation of manual extraction
1.0	November 22, 2022	Initial release

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Chapter 1 Introduction

1.1 Product name

MGIEasy Circulating DNA Extraction Max Kit

1.2 Specifications

Cat. No.	Model	Specification
940-000324-00	CDT-50	50 preps

1.3 Intended use

This kit is used to purify high-quality circulating DNA (cfDNA) from plasma, serum or urine. The purified DNA can be used in downstream applications such as PCR, real-time PCR, biochip analysis, and NGS.

1.4 Working principle

By using this product, salt ions with high concentration lyse and release DNA from plasma, serum or urine. The released DNA is then captured by magnetic beads and washed by a specific wash buffer to remove proteins, salt, and other impurities. After being dried, the DNA in magnetic beads is eluted by the elution buffer and high-purity DNA is obtained.

1.5 Main components

Tips

- Mixed use of reagent components from different batches is not recommended.
- Avoid placing the kit below 0 ℃ to prevent the magnetic beads from being frozen.
- If the reagents have precipitate, it is normal and does not affect the performance of the reagent. Before use, place these reagents in a 50 ℃ water bath, wait for the precipitate to dissolve, and shake thoroughly.

Table 1 MGIEasy Circulating DNA Extraction Max Kit (CDT-50)
Cat. No.: 940-000324-00

Component	Specification	Storage condition	Validity period	Transportation condition
Max Buffer LYS	42.5 mL/bottle	2 ℃ to 30 ℃	12 months	2 ℃ to 30 ℃
Max Binding Buffer	32.5 mL/bottle			
Max Wash Buffer 1-1	50 mL/tube			
Max Wash Buffer 1-2	50 mL/bottle			
Buffer W2	20 mL/bottle			
TE Buffer	2.5 mL/bottle			
Magnetic Beads	3.5 mL/bottle			
Proteinase K	5 mL/bottle			

Chapter 2 Applicable device

Device model	Name	Manufacturer
MGISP-NEXRS	Automated Nucleic Acid Extractor	MGI

Chapter 3 Sample requirements

3.1 Applicable sample

This product is applicable to plasma, serum, and urine.

3.2 Sample storage

Avoid repeatedly freezing and thawing samples, which may result in low DNA quality.

Before use, thaw frozen samples and mix them thoroughly.

3.3 Sample transportation

Use the dry ice for transportation. During transportation, avoid frequent freeze-thaw cycles.

3.4 Sample safety

All samples are regarded potentially infectious. All operations shall be performed in accordance with relevant national standards.

Chapter 4 Operation

4.1 Preparing materials

Prepare the following materials:

Table 2 User-supplied materials for manual extraction

Type	Item	Description
Equipment	Benchtop centrifuge	With a speed no less than 2000 rpm
	Vortex mixer	/
	Metal heater	It can be replaced by a water bath
	1.5 mL, 5 mL, 15 mL, 50 mL Magnetic rack	Depends on your requirement
	Pipette	1 mL, 200 µL, 20 µL
Reagent	Absolute ethanol	Analytically pure
	Isopropanol	Analytically pure
Consumable	1.5 mL, 5 mL, 15 mL, 50 mL centrifuge tube	DNase-free, RNase-free
	Pipette tips	1 mL, 200 µL, 20 µL

Table 3 User-supplied materials for automation extraction

Type	Item	Description
Equipment	MGISP-NEXRS Automated Nucleic Acid Extractor	MGI, Cat. No.: 900-000731-00
	Benchtop centrifuge	With a speed no less than 2000 rpm
	Vortex mixer	/
	Pipette	1 mL, 200 µL, 20 µL
Reagent	Absolute ethanol	Analytically pure
	Isopropanol	Analytically pure
Consumable	Tips	DNase-free, RNase-free

4.2 Preparations

- If Max Buffer LYS, Max Binding Buffer, Max Wash Buffer 1-1, or Max Wash Buffer 1-2 has precipitate, place the reagent in a 50 ℃ water bath for re-dissolve. Shake and mix thoroughly before use.
- All reagents and samples need to be equilibrated to room temperature (10 ℃ to 30 ℃) before use.
- Before use, ensure that 100 mL absolute ethanol has been added into the Buffer W2.
- The sample input volume of 2 mL is recommended. The packing volume of the kit is based on 2 mL sample input volume. If samples of other specifications are extracted and the reagents are not enough, purchase the reagents separately.

4.3 Extracting the nucleic acids manually

Perform the following steps:

1. Add the sample and reagents to a clean 15 mL tube according to the following table. Mix the tube thoroughly with a vortex mixer.

Reagent/sample	Volume
Proteinase K	100 µL
Plasma or serum sample	2 mL
Max Buffer LYS	850 µL

Tips Do not mix Proteinase K directly with Max Buffer LYS.

2. Incubate the tube at 60 ℃ for 20 minutes.
3. Add 650 µL Max Binding Buffer, 1.3 mL isopropanol, and 70 µL Magnetic Beads, and mix them thoroughly by vortex. Incubate the tube at room temperature for 10 minutes, and mix the tube thoroughly for 2-3 times.

Tips
- Mix Magnetic Beads thoroughly before use.
- If the volume of urine sample is 20 mL. Centrifuge the urine samples at 4 ℃ and 4000 g for 10 minutes, and aspirate the supernatant for later use. the amount of each reagent to be added is: 1 mL Proteinase K, 8.5 mL Max Buffer LYS, 6.5 mL Max Binding Buffer, 13 mL Isopropanol, and 70 µL Magnetic Beads.
- You can select the appropriate reaction systems for experiments according to different sample inputs.

Sample (mL)	Centrifuge Tube (mL)	Proteinase K (µL)	Max Buffer LYS (µL)	Max Binding Buffer (µL)	Isopropanol (mL)	Magnetic Bead (µL)
1	5	50	425	325	0.65	35
2	15	100	850	650	1.3	70
3		150	1275	975	1.95	
4		200	1700	1300	2.6	
5	50	250	2125	1625	3.25	
6		300	2550	1950	3.9	
7		350	2975	2275	4.55	
8		400	3400	2600	5.2	
9		450	3825	2925	5.85	
10		500	4250	3250	6.5	

4. Centrifuge the tube immediately and place it on the magnetic rack for 2 to 3 minutes. After the liquid clears, carefully aspirate and remove the supernatant.
5. Remove the tube from the magnetic rack and add 1000 µL of Max Wash Buffer 1-1 into the tube.
6. Transfer the mixture into a new 1.5 mL centrifuge tube and mix the tube thoroughly for 5 to 10 seconds.
7. Centrifuge immediately and place it on the magnetic rack for 1 minute. After the liquid clears, carefully aspirate and remove the supernatant.
8. Remove the tube from the magnetic rack and add 1000 µL of Max Wash Buffer 1-2 into the tube.

9. Mix the tube thoroughly for 5 to 10 seconds and centrifuge immediately.
10. Place it on the magnetic rack for 1 minute. After the liquid clears, carefully aspirate and remove the supernatant.
11. Remove the tube from the magnetic rack. Add 1000 µL Buffer W2 (ensure that absolute ethanol has been added), and mix thoroughly for 5 - 10 seconds.
12. Place the tube on the magnetic rack for 1 minute. After the liquid clears, carefully aspirate and remove the supernatant.
13. Repeat steps 12 to 13 for a second wash for Buffer W2.
14. Keep the tube on the magnetic rack. Open and dry the tube at room temperature for 5 to 7 minutes to ensure that the ethanol is completely evaporated. Do not over-dry the sample. When the surface of the magnetic beads is matte and there is no reflection on the water surface, it means the sample is completely dry.
15. Remove the tube from the magnetic rack and add 50 µL of TE Buffer into the tube.
16. Mix the tube thoroughly with a vortex mixer and place it into a metal heater to incubate it at room temperature at 1000 rpm for 5 minutes.

    Tips The volume of TE Buffer can be adjusted appropriately according to the sample input volume. It ranges between 50 µL and 80 µL.
17. Centrifuge immediately and place it on the magnetic rack for 1 minute. After the liquid clears, carefully transfer the DNA product to a new 1.5 mL tube. Mark and store it at -20 ℃ or below.

4.4 Extracting the nucleic acids with MGISP-NEXRS

4.4.1 Preparing the consumables

Take out the consumables required for one workflow according to the following table and place them at room temperature.

Table 4 User-supplied automation materials

Name	Brand	Cat. No.	Quantity
200 µL Tips, Transparent, Filtered, Suspended	MGI	091-000158-00	1 box
1000 µL Tips, Transparent, Filtered, Suspended	MGI	1000023970	2 boxes

Name	Brand	Cat. No.	Quantity
100 mL Single Well Reservoir	MGI	012-000779-00	4 plates
50 mL Single Well Reservoir	MGI	012-000780-00	2 plates
2.2 mL 96 Well V-bottom Deepwell Plate	MGI	091-000287-00	1 plate
24 Well square deep well plate 10 mL	MGI	091-000442-00	6 plates
24-10 mL Well magnet set	MGI	091-000441-00	1 plate
5 mL tube (with cover)	MGI	100-000052-00	1-24 tubes

4.4.2 Preparing the samples

MGISP-NEXRS can process 1-24 samples at one time. Take out the samples and thaw them at room temperature, centrifuge and mix them thoroughly.

4.4.3 Preparing the reagents

Perform the following steps:

1. Add the absolute ethanol into Buffer W2 according to the label on Buffer W2 bottle.
2. Take out one 24 Well square deep well plate 10 mL (MGI, 091-000442-00), one 2.2 mL 96 Well V-bottom Deepwell Plate, four 100 mL Single Well Reservoirs, and two 50 mL Single Well Reservoirs, mark them. If processing 24 samples, add the corresponding reagents according to the volumes listed in the following table. For other sample quantities, prepare reagents according to the automated operating instruction.

Reagent	Consumable	Volume/well
Sample	5 mL tube (with cover)	2 mL
Max Buffer LYS	50 mL Single Well Reservoir	23 mL
Max Binding Buffer	50 mL Single Well Reservoir	18 mL
Max Wash Buffer 1-1	100 mL Single Well Reservoir	27 mL
Max Wash Buffer 1-2	100 mL Single Well Reservoir	27 mL
Buffer W2	100 mL Single Well Reservoir	54 mL
Isopropyl alcohol	100 mL Single Well Reservoir	35 mL
Proteinase K	Column 1 of 96 Well Plate	330 µL
Magnetic Beads	Column 2 of 96 Well Plate	230 µL

Reagent	Consumable	Volume/well
TE Buffer	Column 3 of 96 Well Plate	160 μL

Tips
- When packing reagents, avoid bubbles at the bottom of reagent wells or reagent remaining on the wall.
- Since the magnetic beads settle very quickly, the beads need to be shaken and mixed thoroughly before use.

4.4.4 Automated extraction

Perform the following steps:

1. Double-click the control software icon on the desktop. The Login interface is displayed.
2. Select **User** and enter the password. Click **Login** to enter the main interface.
3. Click **Workflow** to enter the initialization interface and click **Initialize** .
   The initialization takes about 2 minutes. If **Initialize Successfully** is displayed, it indicates that the device is connected successfully, and you can go to the next step.

   Tips If the initialization fails, check whether the power switch is turned on, and whether more than one software program is running. Try to restart the software. If the problem persists, contact the technical support.
4. Click **Run** .
5. In the Run interface, click **Browse** , select **JB-A58-005 Nucleic acid extraction Kit_RV2.0_SV1.0** , and select the script of **JB-A58-005 Nucleic acid extraction Kit_RV2.0_SV1.0** . The operation deck arrangement is displayed as shown in following figure. Follow the table below to place the samples, reagents, and consumables, confirm the placement, and close the door.

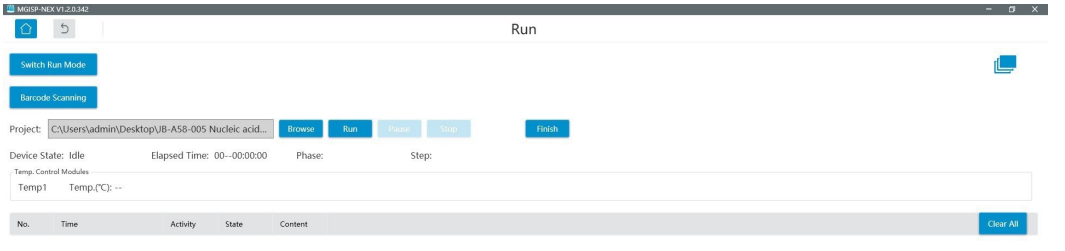

Figure 1 Run interface

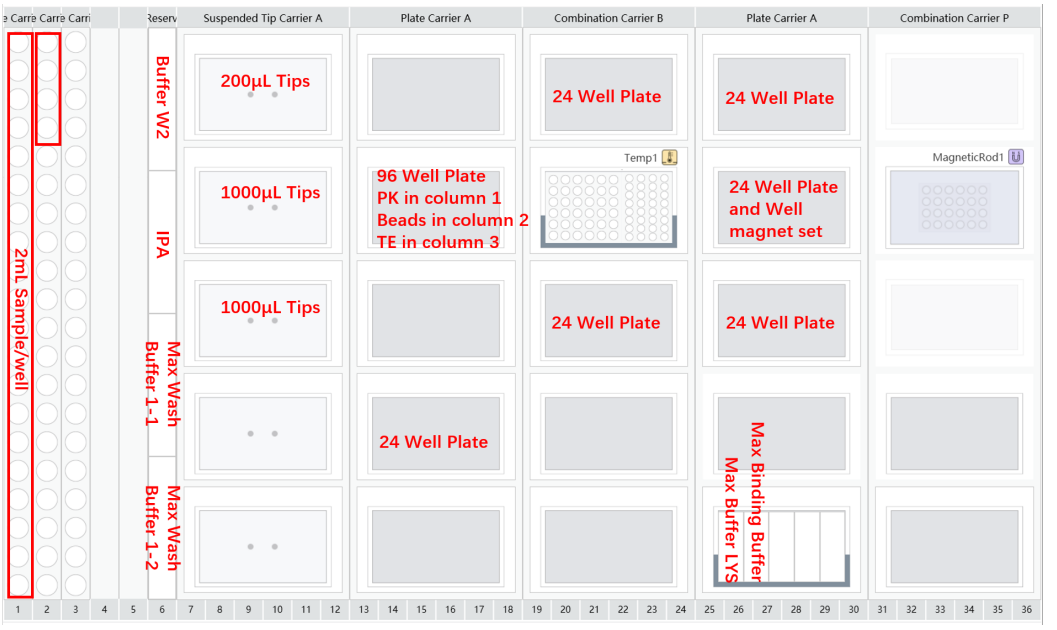

Figure 2 Operation deck arrangement

Table 5 Deck positions of the sample, reagent, and consumables

Name	Consumable	Position
Sample	5 mL tube (with cover)	Lane1, Lane2
200 µL Tips, Conductive, Filtered, Suspended	/	Lane7-POS1
1000 µL Tips, Conductive, Filtered, Suspended	/	Lane7-POS2, Lane7-POS3
Buffer W2	100 mL Single Well Reservoir	Lane6-POS1
Isopropyl alcohol	100 mL Single Well Reservoir	Lane6-POS2
Max Wash Buffer 1-1	100 mL Single Well Reservoir	Lane6-POS3
Max Wash Buffer 1-2	100 mL Single Well Reservoir	Lane6-POS4
Max Buffer LYS	50 mL Single Well Reservoir	Lane25-POS5-1
Max Binding Buffer	50 mL Single Well Reservoir	Lane25-POS5-2

Name	Consumable	Position
Proteinase K +Magnetic Beads+TE Buffer	2.2 mL 96 Well V-bottom Deepwell Plate	Lane13-POS2
Sample	24 Well square deep well plate 10 mL	Lane25-POS2
24 Well square deep well plate 10 mL	/	Lane13-POS4, Lane19-POS1, Lane19-POS3, Lane25-POS1, Lane25-POS2, Lane25-POS3

6. Click **Run** . A window pops up. Click **OK** .

Figure 3 Confirmation window

7. Click the **Please select sample number** list in the window and click **Continue** .

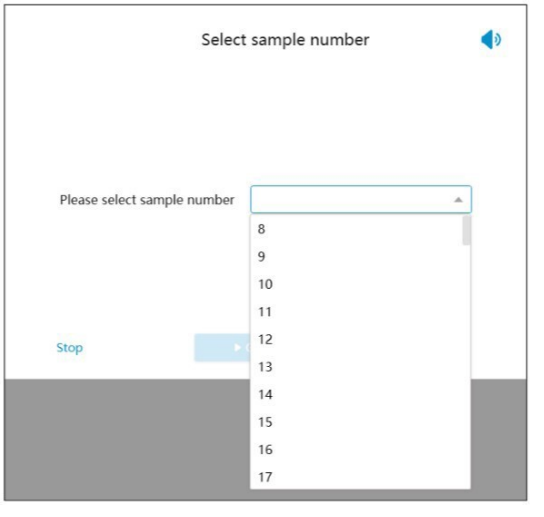

Figure 4 Sample number selection window

8. The workflow takes about 90 minutes. After the workflow is completed, take out the product at Lane13-POS4 and store it at -20 ℃ or below.

9. Dispose of the used deep-well plates, PCR plates, and waste bag to the designated waste area. Perform a post-clean according to *MGISP-NEX Cleaning Instructions* .

Chapter 5 Warnings and precautions

- This product is for research use only and should not be used for clinical diagnosis. 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.
- Please confirm the following issues before starting the experiment, otherwise it will influence the extracting result:
  - Ensure that all reagents are stored in the recommended conditions.
  - Ensure that there is no ethanol residue after ethanol washing.
  - Ensure that Heparin anticoagulated plasma is not used. Otherwise, it will affect the quality of extracted nucleic acid.
  - Ensure that the plasma or serum is not frozen or thawed for more than 3 times.
  - Ensure that hemolysis or coagulation samples are not used. Otherwise, it will affect the extraction efficiency.
  - Ensure that the micro-pipette is used to add samples.
- Mix reagents thoroughly before use.
- 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.

Appendix 1 Manufacturer information

Manufacturer	Wuhan MGI Tech Co., Ltd.
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