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Research Use Only

Wuhan MGI Tech Co., Ltd.

MGIEasy

Magnetic Beads Genomic **DNA** Extraction Kit

Instructions for Use

Version: 3.0

About the instructions for use

This instructions for use is applicable to MGIEasy Magnetic Beads Genomic DNA Extraction Kit. The version of the instructions for use is 3.0 and the kit version is 1.0.

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Revision history

| Version | Date | Description |
|---------|-------------------|---|
| 3.0 | June 20, 2024 | Added the information on the dried blood spot samples |
| 2.0 | December 26, 2023 | Revised 4.3.1Revised the script name for extraction on MGISP-NE384RS |
| 1.0 | August 15, 2023 | Initial release |

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Chapter 1 Introduction

1.1 Product name

MGIEasy Magnetic Beads Genomic DNA Extraction Kit

1.2 Specifications

| Kit name | Model | Cat. No. | Specification |
|--|---------|---------------|---------------|
| MGIEasy Magnetic Beads Genomic DNA Extraction Kit | WDT-96 | 940-000972-00 | 96 Preps |
| MGIEasy Magnetic Beads Genomic DNA Extraction Kit | WDT-864 | 940-000973-00 | 864 Preps |

1.3 Intended use

This set is used to extract, enrich and purify nucleic acids.

1.4 Working principle

By using the unique, high-binding, super-paramagnetic beads, this kit is used to extract high-quality genomic DNA quickly and easily from blood, saliva stored by MGI saliva DNA collection kit, fresh saliva, buccal swabs, animal tissues, cells, dried blood spots and other samples. The extracted genomic DNA can be used for various routine applications, including enzyme digestion, PCR, real-time PCR, library preparation, chip hybridization and high-throughput sequencing.

1.5 Main components

Tips • Do not mixedly use reagents from different batches of kits.

- Store the kit in a dry environment. To store Proteinase K and Magnetic Beads H for a longer time, store these two reagents in a refrigerator at 2 °C to 8 °C.
- That precipitation forms in Buffer LB and Buffer W1 is normal and does not affect the reagent performance. Before use, preheat the reagents for 10 minutes in a water bath at 37 °C to dissolve the precipitation and mix the reagents thoroughly.
- Before use, take out all components in the reagent kit, equilibrate to room temperature (10 °C to 30 °C) and mix them thoroughly before adding to wells.
- Buffer EB consists of 10 mM Tris-HCl (pH 8.0) and 0.5 mM EDTA (pH 8.0). Please prepare the elution buffer according to your specific needs.

Table 1 MGIEasy Magnetic Beads Genomic DNA Extraction Kit (WDT-96) Cat. No.: 940-000972-00

| Name | Component | Specification | Storage condition | Validity period | Transportation condition |
|--|---------------------|----------------|-----------------------|--------------------|--------------------------|
| MGIEasy Magnetic Beads Genomic DNA Extraction Kit Cat. No.: 940-000972-00 | Buffer LS | 22 mL/bottle×1 | 2 ℃ to 30 ℃ 18 months | 18 months | 2 ℃ to 30 ℃ |
| | Buffer LB | 30 mL/bottle×1 | | | |
| | Buffer W1 | 28 mL/bottle×1 | | | |
| | Buffer W2 | 30 mL/bottle×1 | | | |
| | Buffer EB | 20 mL/bottle×1 | | | |
| | Proteinase K | 2 mL/tube×1 | | | |
| | Magnetic Beads H | 2 mL/tube×1 | | | |

| Name | Component | Specification | Storage condition | Validity period | Transportation condition |
|--|---------------------|-----------------|----------------------|--------------------|--------------------------|
| MGIEasy Magnetic Beads Genomic DNA Extraction | Buffer LS | 200 mL/bottle×1 | 2 ℃ to 30 ℃ 18 r | 18 months | 2 ℃ to 30 ℃ |
| | Buffer LB | 260 mL/bottle×1 | | | |
| | Buffer W1 | 240 mL/bottle×1 | | | |
| | Buffer W2 | 140 mL/bottle×2 | | | |
| Kit Cat. No.: 940-000973-00 | Buffer EB | 180 mL/bottle×1 | | | |
| Cat. NO.: 940-000975-00 | Proteinase K | 18 mL/bottle×1 | | | |
| | Magnetic Beads H | 18 mL/bottle×1 | | | |

Table 2 MGIEasy Magnetic Beads Genomic DNA Extraction Kit (WDT-864) Cat. No.: 940-000973-00

Chapter 2 Applicable device

MGISP-NE384RS Automated Nucleic Acid Extractor

Chapter 3 Sample requirements

3.1 Applicable sample

This product is applicable to blood, saliva stored by MGI saliva sample collection kit, fresh saliva, buccal swabs, amniotic fluid, cells, animal tissues and dried blood spots.

| 3.2 | Sample | amount | requirements |
|-----|--------|--------|--------------|
|-----|--------|--------|--------------|

| | | Sample amount | | |
|---------|--|---------------------------------|---------------------------------|--|
| Sample | type | Manual extraction | Extraction on MGISP-NE384RS | |
| | Fresh/frozen blood | 200 µL | 200 µL | |
| Blood | Anticoagulant blood of poultry, birds, amphibians, or lower organisms | 5 μL to 10 μL | 5 μL to 10 μL | |
| Saliva | Saliva/buccal swab stored by MGI saliva sample collection kit | 500 µL | 500 µL | |
| | Fresh saliva | 200 µL | 200 µL | |
| Cell | | ≤5×10 ⁶ | ≤5×10 ⁶ | |
| Amniot | ic fluid | 3 mL to 5 mL | 3 mL to 5 mL | |
| Animal | tissue | 2 mg to 50 mg | 5 mg to 15 mg | |
| Dried b | lood spots | 3 to 5 pieces, 3 mm in diameter | 3 to 5 pieces, 3 mm in diameter | |

3.3 Sample storage

- For samples of blood, amniotic fluid, cell and animal tissue that could be tested within 24 hours, store them at 2 °C to 8 °C. For those that could not be tested within 24 hours, store them at -70 °C or below, or in a freezer at -25 °C to -15 °C. During storage, do not freeze and thaw samples frequently.
- For the fresh saliva sample, use it immediately after collection. It is recommended to use the MGI saliva sample collection kit (MGI, Cat. No.: 940-001262-00/1000025954) to collect saliva samples which then could be stored at room temperature.
- For dried blood spot samples, store it at room temperature after sampling.
- Do not freeze and thaw frozen samples frequently. Otherwise, the DNA quality may decrease.

3.4 Sample transportation

- For samples of blood, amniotic fluid, cell and animal tissue, use the dry ice for transportation for up to 7 days. During transportation, avoid frequent freeze-thaw cycles.
- For samples stored by MGI saliva sample collection kit or dried blood spot samples, transport them at room temperature.

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

| Туре | Item | Description |
|-----------|--|--|
| | MGISP-NE384RS automated nucleic acid extractor | MGI, Cat. No.: 900-000357-00For use in automated extraction |
| | Mini centrifuge | With a speed no less than 12000 rpm |
| Equipment | Vortex mixer | None |
| | Thermomixer compact | It can be replaced by a water bath |
| | 1.5 mL magnetic rack | None |
| | Pipette | 1 mL/200 μL/20 μL/10 μL |
| | Absolute ethanol | Analytically pure |
| Reagent | Isopropanol | Analytically pure |
| | RNase A | 20 mg/mL DNase-free |

| Туре | Item | Description |
|-------------|------------------------------|------------------------------|
| | Colina comple collection kit | MGI, Cat. No.: 940-001262-00 |
| | Saliva sample collection kit | MGI, Cat. No.: 1000025954 |
| Consumables | Tips | 1 mL/200 μL/20 μL/10 μL |
| | Contrifu an tube | • 5 mL/2 mL/1.5 mL |
| | Centrifuge tube | DNase-free and RNase-free |

4.2 Pretreating samples

Pretreat samples according to different types of samples. In automated extraction, there is no need to pretreat blood and saliva samples.

Tips Please thaw and mix the frozen samples thoroughly before use.

4.2.1 Blood sample

Perform the following steps:

- 1. Perform different steps according to the blood type.
 - For fresh or frozen blood, add 200 µL of sample into a new 1.5 mL centrifuge tube.
 - For anticoagulant blood of poultry, birds, amphibians, or lower organisms, add 5 μL to 10 μL of sample into a new 1.5 mL centrifuge tube.
- 2. Add Buffer LS into the tube to bring the final volume to 200 μL if the total volume is less than 200 $\mu L.$
- 3. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 4. Add 300 μL of Buffer LB into the tube, and mix the tube thoroughly by vortexing.

4.2.2 Saliva sample

Perform the following steps:

- 1. Perform different steps according to the saliva type.
 - For saliva or buccal swab stored by MGI saliva sample collection kit, add 500 µL of sample into a new 1.5 mL centrifuge tube.
 - Tips Mix the sample thoroughly before sampling to obtain sufficient DNA sample.

- For fresh saliva sample, add 200 µL of sample into a new 1.5 mL centrifuge tube.
 - **Tips** If the sample is not extracted immediately, add 300 µL of Buffer LB into the tube, mix thoroughly and store it at room temperature. Perform the next step within 24 hours.
- 2. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 3. Perform different steps according to the saliva type.
 - For saliva or buccal swab stored by MGI saliva sample collection kit, or fresh saliva stored by Buffer LB, go to the next step directly.
 - For fresh saliva sample, add 300 µL of Buffer LB into the centrifuge tube and mix the tube thoroughly by vortexing.

4.2.3 Cell sample

Perform the following steps:

- 1. Add cell suspension sample whose extraction volume does not exceed 5×10⁶ into a new 1.5 mL centrifuge tube.
 - For cell suspension sample with high concentration, add Buffer LS to dilute the sample to that of less than 5×10⁶ cells/mL.
 - For adherent cells, perform the following steps:
 - a. Prepare cell suspension from sample. Add 1 mL of sample into a new 1.5 mL centrifuge tube.
 - b. Centrifuge the tube in a centrifuge at 10000 rpm for 1 minute.
 - c. Remove the supernatant, add 200 μL of Buffer LS into the tube, and vortex it to suspend it completely.
- 2. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 3. Place the tube into a thermomixer compact to incubate it for 30 to 60 minutes at 65 °C with a speed of 1000 rpm to 1200 rpm. When the solution is transparent without visible turbidity, briefly centrifuge the tube and ensure that no precipitate exists at the bottom of the tube.
- 4. Add 300 μL of Buffer LB into the centrifuge tube and mix the tube thoroughly by vortexing.

4.2.4 Amniotic fluid sample

Perform the following steps:

- 1. Add 3 mL to 5 mL of amniotic fluid sample into a new 5 mL centrifuge tube.
- 2. Centrifuge the tube in a centrifuge at 6000 rpm for 2 minutes.
- 3. Remove the supernatant without aspirating the pellet.
- 4. Add Buffer LS into the tube to bring the final volume to 200 μ L. Transfer the reagent in the tube to a new 1.5 mL centrifuge tube.
- 5. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 6. Place the tube into a thermomixer compact to incubate it for 30 to 60 minutes at 65 °C with a speed of 1000 rpm to 1200 rpm. When the solution is transparent without visible turbidity, briefly centrifuge the tube and ensure that no precipitate exists at the bottom of the tube.
- 7. Add 300 μL of Buffer LB into the centrifuge tube and mix the tube thoroughly by vortexing.

4.2.5 Animal tissue sample

Perform the following steps:

- Prepare 2 mg to 50 mg (manual extraction) or 5 mg to 15 mg (extraction on MGISP-NE384RS) of fresh or frozen tissue sample, use a surgical knife or a pair of surgical scissors to cut the sample as big as a sesame seed and then add them into a new 1.5 mL centrifuge tube.
- 2. Add 200 μ L of Buffer LS into the tube, and vortex it to suspend it completely.
- 3. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 4. Place the tube into a thermomixer compact to incubate it for 30 to 60 minutes at 65 °C with a speed of 1000 rpm to 1200 rpm. When the solution is transparent without visible turbidity, briefly centrifuge the tube and ensure that no precipitate exists at the bottom of the tube.
- 5. Add 300 μL of Buffer LB into the centrifuge tube and mix the tube thoroughly by vortexing.

4.2.6 Dried blood spot sample

Perform the following steps:

1. Prepare 3 to 5 pieces of dried blood spot samples being 3 mm in diameter and add them into a new 2 mL centrifuge tube.

- 2. Add 200 μ L of Buffer LS into the tube, and vortex it to suspend it completely.
- 3. Add 20 μL of Proteinase K into the tube, and mix the tube thoroughly by vortexing.
- 4. Place the tube into a thermomixer compact to incubate it for 60 minutes at 65 °C with a speed of 1000 rpm to 1200 rpm.
- 5. Briefly centrifuge the tube, add 300 μ L of Buffer LB into the centrifuge tube and mix the tube thoroughly by vortexing.
- 6. Place the tube into a thermomixer compact to incubate it for 15 minutes at $65 \,^\circ C$ with a speed of 1000 rpm to 1200 rpm.
- 7. Centrifuge the tube for 1 minute at 12000 rpm in a centrifuge.
- 8. Transfer 500 µL of supernatant into a new 1.5 mL centrifuge tube (manual extraction) or a deep-well plate (extraction on MGISP-NE384RS).

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 have prepared applicable consumables.

4.3.1 Extracting the nucleic acids manually

Before extraction, add absolute ethanol into Buffer W1 and Buffer W2 according to the label.

Perform the following steps:

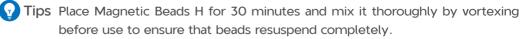
1. Place the tube into a thermomixer compact to incubate it for 15 minutes at 65 °C with a speed of 1000 rpm to 2000 rpm.

Y Tips For dried blood spot samples, skip this step and start from the next step.

2. Add 350 μ L of isopropanol or 400 μ L of isopropanol (only for dried blood spots) into the tube and mix the tube thoroughly by vortexing. It is normal that the flocculent precipitation forms in the tube.

Tips For dried blood spot samples, you need to add 400 µL of isopropanol.

3. Add 20 µL of Magnetic Beads H into the tube and mix the tube thoroughly by vortexing. Place the tube at room temperature for 5 minutes during which vortex it every 2 minutes.



- 4. Centrifuge the tube briefly and place it on the magnetic rack for 2 minutes. When Magnetic Beads H is adsorbed completely, use a pipette to slowly remove the supernatant.
- 5. Remove the tube from the rack. Add 500 μ L of Buffer W1 and mix the tube thoroughly by vortexing for 1 to 2 minutes.

Tips Please do mix the tube thoroughly. Otherwise, the DNA purity may be affected.

- 6. Place the tube on the magnetic rack for 1 minute. When Magnetic Beads H is adsorbed completely, use a pipette to slowly remove the supernatant.
- 7. Remove the tube from the rack. Add 600 μ L of Buffer W2 and mix the tube thoroughly by vortexing for 1 to 2 minutes.
- 8. Place the tube on the magnetic rack for 1 minute. When Magnetic Beads H is adsorbed completely, use a pipette to slowly remove the supernatant.
- 9. Repeat step 7 to 8 once. During operation, remove the remaining liquid in the tube as much as possible.
- 10. Place the tube on the magnetic rack, and decap and dry the tube at room temperature for 5 to 10 minutes until the beads are not reflecting or cracking. At this time, the ethanol evaporates completely.
- 11. Remove the tube from the rack. Add 50 μ L to 100 μ L of Buffer EB into the tube and mix the tube thoroughly by vortexing. Place the tube into a thermomixer compact to incubate it for 5 minutes at 56 °C with a speed of 1000 rpm.
 - **Tips** To remove RNA, add Buffer EB containing RNase A into the tube at this step. It is recommended to add 0.5 μL of RNase A (20 mg/mL) for every 100 μL of Buffer EB.
- 12. Place the tube on the magnetic rack. When Magnetic Beads H is adsorbed completely, transfer the DNA solution into a new 1.5 mL centrifuge tube. Mark the tube and store it at -20 °C or below.
 - **Tips** The recommended volume of Buffer EB is no less than 50 μ L.
 - When DNA yield is greater than normal, DNA is not completely dissolved and thus magnetic beads may be adhered by viscous liquid. You need to add an appropriate volume of Buffer EB to completely dissolve it. If beads are still adhered, centrifuge the tube in a centrifuge at 8000 rpm for 1 minute.

4.3.2 Extracting the nucleic acids automatically on MGISP-NE384RS

4.3.2.1 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. | Number |
|---|-------|------------|--------|
| 2.2 mL V-bottom 96-well deep-well plate | MGI | 1000008088 | 20 |
| 96-well tips comb | MGI | 1000025661 | 4 |

4.3.2.2 Preparing samples

You can extract 1 to 384 samples on MGISP-NE384RS.

Perform the following steps:

- 1. Take out a deep-well plate and mark it as 'sample'.
- 2. Perform different steps according to the sample type.
 - For samples of blood, amniotic fluid, cell, animal tissue and dried blood spot, ensure that the sample is pretreated according to *Pretreating samples* on Page 6 and add the sample into the plate for sample.
 - For other samples, add the sample and other reagents directly into the plate according to the following table.

| Pargont | Adding volume for each well (μL) | | | |
|-----------------|---|---|--------------------------------------|--|
| Reagent name | Whole blood/ fresh saliva | Blood of poultry, birds, or amphibians | Saliva (with saliva preservative) | |
| Sample | 200 | V (5 to 10) | 500 | |
| Buffer LS | / | 200-V | / | |
| Proteinase K | 20 | 20 | 20 | |
| Buffer LB | 300 | 300 | / | |

3. Place the plate on ice until use.

4.3.2.3 Preparing reagents

Perform the following steps:

1. Add absolute ethanol into Buffer W1 according to the label.

- 2. Add absolute ethanol into Buffer W2 according to the label.
- 3. Take out 4 96-well deep-well plates. Add reagents according to the following table and mark the plates for each type of reagent.

| Reagent name | Adding volume |
|--------------|-------------------------------------|
| Buffer W1 | 500 µL/well |
| Buffer W2 | 600 µL/well |
| Buffer W2 | 600 µL/well |
| Buffer EB | 60 $\mu L/well$ to 150 $\mu L/well$ |

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 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. Empty the operation deck and close the door.
- 6. Select Clean in the main interface.
- 7. Click **Start**. The default duration is 20 minutes and you can also set the time as required.

UV lamps are turned on and air filter starts working.

CAUTION The ultraviolet radiation is harmful to the human body, so do not open the door after the cleaning starts.

- 8. Click **Process manage**> 💽 to import the script.
- 9. Click Source Strategies Workflow. Click the drop-down list of Script and select MGIEasy Genomic DNA Extraction Kit_V1.0.mgi. Place samples, reagents and consumables according to the following table:

| Reagent name | Position |
|--------------|----------|
| Sample | Pos1 |
| Buffer W1 | Pos2 |
| Buffer W2 | Pos3 |

| Reagent name | Position |
|--------------|----------|
| Buffer W2 | Pos4 |
| Buffer EB | Pos6 |

- 10. Place 96-well tips comb according to the sample number.
- 11. Click **Run**. Select the required lanes and tips comb in the pop-up window. Click **OK**. The device starts extraction according to the following table. The whole workflow takes about 40 minutes.
 - **Tips** For dried blood spot samples, you need to modify the script manually, including setting the mixing time in step 2 to 10 seconds, setting the prompt to remind users to add 400 μL of isopropanol and 20 μL of Magnetic Beads H to each sample well of Pos1 plate, and setting the temperature of Pos1 in the temperature control settings at 25 °C.

| | Step 1 | Step 2 | Step 3 | Step 4 | Step 5 | Step 6 | Step 7 | Step 8 |
|-------------------------|--------|--|--------|--------|--------|--------|---------|---------|
| Step name | Lysis | Lysis | Bind | Wash | Wash | Wash | Elution | Release |
| Position | 5 | 1 | 1 | 2 | 3 | 4 | 6 | 2 |
| Volume (µL) | 520 | 520 | 870 | 500 | 600 | 600 | 100 | 500 |
| Delay time (s) | 0 | 0 | 0 | 0 | 0 | 0 | 120 | 0 |
| Mix | False | True | True | True | True | True | True | True |
| Mix type | / | Magnetic | Normal | Normal | Normal | Normal | Normal | Normal |
| Mix rate | Middle | Middle | Middle | High | High | High | High | High |
| Mix time (s) | 1 | Dried blood spot samples: 10 Other samples: 900 | 180 | 180 | 120 | 120 | 300 | 5 |
| Collect | True | False | True | True | True | True | True | False |
| Collect mode | Normal | Normal | Cycle | Cycle | Cycle | Cycle | Cycle | Normal |
| Collect cycle (time) | 1 | 1 | 2 | 2 | 2 | 2 | 10 | 1 |
| Collect time (s) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Dialog | False | True | False | False | False | False | False | False |

| | Step 1 | Step 2 | Step 3 | Step 4 | Step 5 | Step 6 | Step 7 | Step 8 |
|-------------------|--------|---|--------|--------|--------|--------|--------|--------|
| Dialog content | / | Dried blood spot samples: Add 400 µL of isopropanol and 20 µL of Magnetic Beads H to each sample well of Pos1 plate. Other samples: Add 350 µL of isopropanol and 20 µL of Magnetic Beads H to each sample well of Pos1 plate. | / | / | / | / | / | / |

Before step 3, you will be prompted to confirm that you have added 350 μ L of isopropanol or 400 μ L of isopropanol (only for dried blood spot samples), and 20 μ L of Magnetic Beads H to each sample well of the Pos1 plate. Click **OK** and step 3 starts.

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

| Position | Pos1 | Pos6 | | |
|-------------|---|-------|--|--|
| Temperature | Dried blood spot samples: 25 °C Other samples: 75 °C | 56 °C | | |
| Open step | Step1 | Step7 | | |
| Close step | Step2 | Step7 | | |
| Action | Mix | Mix | | |
| Order | After | After | | |

The temperature control settings are as follows:

- 12. After the program ends, transfer the 96-well tips comb to the medical waste bag.
- 13. Immediately remove the 96-well plate from Pos6, seal the plate and store it in a freezer at -20 $^\circ\!\mathrm{C}$.

You can also transfer the DNA product in the 96-well plate from Pos6 to a new plate, seal and store it in a freezer at -20 $^\circ\!C$.

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.
- You should prepare the isopropanol and RNase A (20 mg/mL) before the experiment.
- Please use recommended consumables for experiment.
- After the experiment, ensure that bottle caps, particularly caps of Buffer W1 and Buffer W2 containing the absolute ethanol, are securely tightened.
- Buffer EB consists of 10 mM Tris-HCl (pH 8.0) and 0.5 mM EDTA (pH 8.0). Please prepare the elution buffer according to your specific needs.
- 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. |
|-------------------|---|
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