Part No.: H-T-098

MGIEasy

Circulating DNA Extraction Mini Kit (CDT-48 & CDT-192)

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 Circulating DNA Extraction Mini Kit (CDT-48 & CDT-192). 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	November 11, 2024	1. Revised the position of the magnetic rack to Pos15 in Table 4 2. Revised the storage temperature of the extraction product to -20 ℃ in 4.3 3. Revised to “can process 1-16 samples” in 4.5.2
2.0	July 30, 2024	1. Revised the kit components 2. Revised the operation of manual extraction 3. Added steps for MGISP-960RS extraction 4. Deleted steps for MGISP-NE384RS extraction 5. Added steps for MGISP-100RS extraction
1.0	November 22, 2022	Initial release

Contents

Chapter 1 Introduction

1.1 Product name

MGIEasy Circulating DNA Extraction Mini Kit

1.2 Specifications

Kit name	Model	Cat. No.	Specification
MGIEasy Circulating DNA Extraction Mini Kit	CDT-48	940-000320-00	48 preps
	CDT-192	940-000318-00	192 preps

1.3 Intended use

This kit is used to purify high-quality circulating DNA (cfDNA) from plasma or serum. 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 or serum. 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 37 ℃ water bath for 10 minutes, wait for the precipitate to dissolve, and shake thoroughly.

Table 1 MGIEasy Circulating DNA Extraction Mini Kit (CDT-48)
Cat. No.: 940-000320-00

Component	Specification	Storage condition	Validity period	Transportation condition
Mini Buffer LYS	14 mL/bottle	2 ℃ to 30 ℃	12 months	2 ℃ to 30 ℃
Mini Wash Buffer 1-1	36 mL/bottle			
Mini Wash Buffer 1-2	36 mL/bottle			
Buffer W2	9.6 mL/bottle			
TE Buffer	2.4 mL/tube			
Magnetic Beads	0.8 mL/tube			
Proteinase K	0.72 mL/tube			

Table 2 MGIEasy Circulating DNA Extraction Mini Kit (CDT-192)
Cat. No.: 940-000318-00

Component	Specification	Storage condition	Validity period	Transportation condition
Mini Buffer LYS	56 mL/bottle	2 ℃ to 30 ℃	12 months	2 ℃ to 30 ℃
Mini Wash Buffer 1-1	144 mL/bottle			
Mini Wash Buffer 1-2	144 mL/bottle			
Buffer W2	38.4 mL/ bottle			
TE Buffer	9.6 mL/bottle			
Magnetic Beads	3 mL/bottle			
Proteinase K	3 mL/bottle			

Chapter 2 Applicable device

Device model	Name	Manufacturer
MGISP-960RS	High-throughput Automated Sample Preparation System	MGI
MGISP-100RS	DNA Sequencing Library Preparation System	

Chapter 3 Sample requirements

3.1 Applicable sample

This product is applicable to plasma and serum.

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 3 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 Magnetic rack	/
	Pipette	1 mL, 200 μL, 20 μL
Reagent	Absolute ethanol	Analytically pure
	Isopropanol	Analytically pure
Consumable	1.5 mL, 2 mL, 5 mL Centrifuge tube	DNase-free, RNase-free
	Pipette tips	DNase-free, RNase-free

Table 4 User-supplied materials for automation extraction

Type	Item	Description
Equipment	MGISP-960RS High-throughput Automated Sample Preparation System	• MGI, Cat. No.: 900-000146- 00 • Device configuration: ▪ Vortex mixer: 1 (Pos20) ▪ Magnetic rack: 1 (Pos15)
	MGISP-100RS DNA Sequencing Library Preparation System	MGI, Cat. No.: 900-000206-00
	Benchtop centrifuge	With a speed no less than 2000 rpm
	Vortex mixer	/
	Plate centrifuge	/
	Pipette	1 mL, 200 μL, 20 μL

Type	Item	Description
Reagent	Absolute ethanol	Analytically pure
	Isopropanol	Analytically pure
Consumable	Sealing film	/
	Tips	DNase-free, RNase-free

4.2 Preparations

- If Mini Buffer LYS, Mini Wash Buffer 1-1 or Mini Wash Buffer 1-2 has precipitate, place the reagent in a 37 ℃ 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 the absolute ethanol has been added into the Buffer W2.

Reagent	Ethanol	
	CDT-48	CDT-192
Buffer W2	40 mL	160 mL

- Use the consumables that are compatible with the automation requirements to prepare the reagents.
- The recommended volume for sample input is 300 µL. The packing volume of the kit is based on 300 µL 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. Mix the tube thoroughly with a vortex mixer.

Reagent/sample	Volume
Proteinase K	15 µL
Plasma or serum	300 µL
Mini Buffer LYS	290 µL

Reagent/sample	Volume
Isopropanol	175 µL
Magnetic Beads	15 µL

Tips
- Do not mix Proteinase K directly with Mini Buffer LYS.
- Mix Magnetic Beads thoroughly before use.

2. Incubate the tube at room temperature for 10 minutes, and vortex 2 or 3 times during this period to avoid beads pellet.

Tips You can select the appropriate reaction systems for experiments according to different sample inputs.

Sample (µL)	Centrifuge Tube (mL)	Proteinase K (µL)	Mini Buffer LYS (µL)	Isopropanol (µL)	Magnetic Beads (µL)
100	1.5	5	97	58	5
200		10	193	117	10
300		15	290	175	15
400		20	387	233	20
500		25	483	292	25
600	2	30	580	350	
700		35	677	408	
800	5	40	773	467	35
900		45	870	525	
1000		50	967	583	

3. 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.
4. Remove the tube from the magnetic rack and add 750 µL of Mini Wash Buffer 1-1 into the tube. Mix the tube thoroughly for 5 to 10 seconds and centrifuge immediately.

Tips If a 5 mL centrifuge tube is selected in step 1, transfer the mixture into a new 1.5 mL centrifuge tube before vortexing.

5. Place the tube on the magnetic rack for 1 minute. After the liquid clears, carefully aspirate and remove the supernatant.
6. Remove the tube from the magnetic rack and add 750 µL of Mini Wash Buffer 1-2 into the tube. Mix the tube thoroughly for 5 to 10 seconds and centrifuge immediately.

7. 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 750 µL of Buffer W2 (ensure that the absolute ethanol is added) 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. 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.
12. Remove the tube from the magnetic rack and add 50 µL of TE Buffer into the tube.
13. 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.

14. 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. Label and store it at -20 ℃ or below.

4.4 Extracting the nucleic acids with MGISP-960RS

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.

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	5 Boxes
1.3 mL U-bottom deep-well plate	MGI	1000004644	7 Plates
Hard-shell thin-wall 96-well skirted PCR plates, white shell/ clear well	MGI	1000012059	2 Plates

4.4.2 Preparing the samples

MGISP-960RS can process 96 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. Mix Magnetic Beads and Isopropanol in a ratio of 3:35, and shake to mix.
3. Take out seven 1.3 mL U-bottom deep-well plates (MGI, 1000004644), two Hard-shell thin-wall 96-well skirted PCR plates, white shell/clear well (MGI, 1000012059), mark them, and add the corresponding reagents according to the volumes listed in the following table.

Marked name	Consumable	Volume/well
Sample	1.3 mL U-bottom deep-well plate	300 µL
Mini Buffer LYS	1.3 mL U-bottom deep-well plate	310 µL
Mini Wash Buffer 1-1	1.3 mL U-bottom deep-well plate	770 µL
Mini Wash Buffer 1-2	1.3 mL U-bottom deep-well plate	770 µL
Buffer W2	1.3 mL U-bottom deep-well plate	770 µL
TE Buffer	1.3 mL U-bottom deep-well plate	60 µL
Magnetic Beads+ IPA	1.3 mL U-bottom deep-well plate	210 µL
Proteinase K	Hard-shell thin-wall 96-well skirted PCR plate	20 µL
cfDNA	Hard-shell thin-wall 96-well skirted PCR plate	/

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 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-tap  to run the software.
3. Select **User** and **Real** . Enter the password.
4. Tap **Login** to enter the main interface.
5. On the upper-right corner of the control software, tap  and select **WDesigner** . The home interface is displayed.
6. Ensure that the application file in the *.wfex* format has been prepared.
7. Tap  in the toolbar and find the file location in the pop-up window.
8. Select the file and tap **Open** , fill in the **Application** and **Project** , and tap **Confirm** to save the application file. Then this application file can be executed in the control software.
9. After the file is imported successfully, tap  in the toolbar.
10. Tap **Initialize** on the top of the interface to start initializing. You will be prompted after a successful initialization.
11. Tap  on the left of the interface, and select **Clean** > **Pre-clean** > **Start** .
12. Follow the on-screen instructions to complete operations and tap **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. Tap  > **Run Wizard** to enter the Run Wizard interface.
14. Tap the drop-down list of **Solution** and select **JB-A09-129 Nucleic acid extraction Kit_RV2.0_SV2.0** . Tap the drop-down list of **Script** , select **JB-A09-129 Nucleic acid extraction Kit_RV2.0_SV2.0** . Place samples, reagents and consumables according to the following table and figure, and close the door.

    WARNING Place the automated pipette tips with the labels facing outward. Ensure that no bubbles exist at the bottom of the reagent plate and sample plate, and no liquid exists on the wall of the plates.

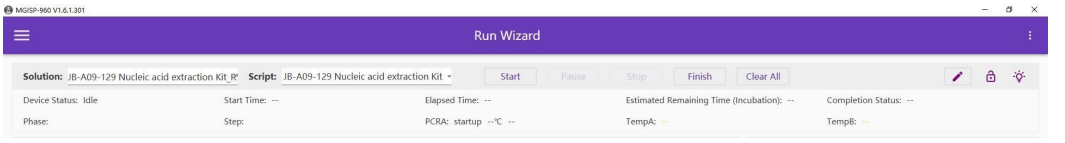

Figure 1 Run Wizard interface

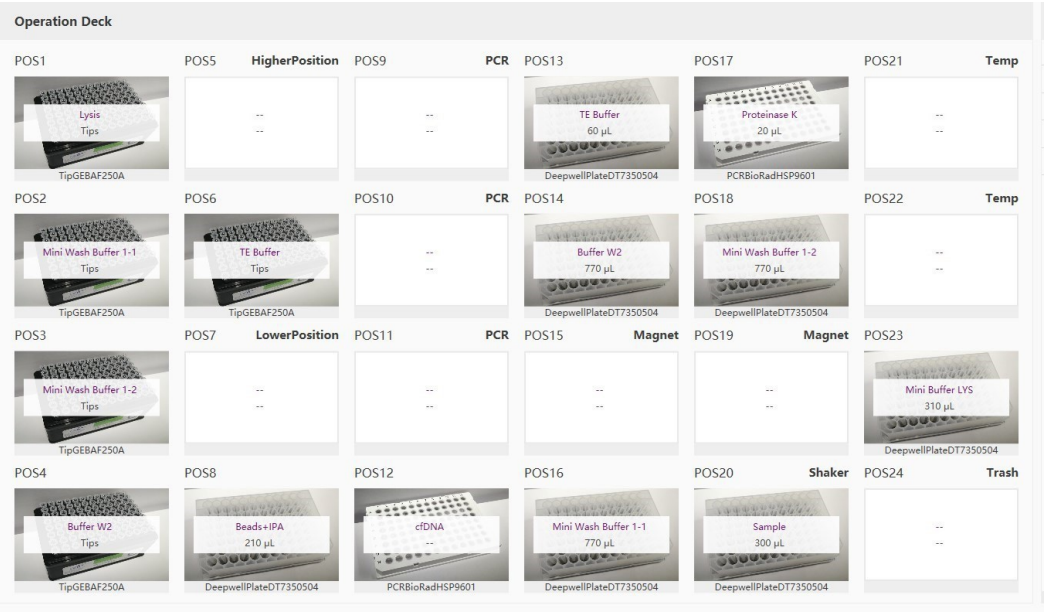

Figure 2 Operation deck arrangement

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

Name	Consumables	Position
250 µL automated filter tips	/	Pos1-Pos4, Pos6
Sample	1.3 mL U-bottom deep-well plate	Pos20
Mini Buffer LYS	1.3 mL U-bottom deep-well plate	Pos23
Mini Wash Buffer 1-1	1.3 mL U-bottom deep-well plate	Pos16
Mini Wash Buffer 1-2	1.3 mL U-bottom deep-well plate	Pos18
Buffer W2	1.3 mL U-bottom deep-well plate	Pos14
TE Buffer	1.3 mL U-bottom deep-well plate	Pos13
Magnetic Beads+ IPA	1.3 mL U-bottom deep-well plate	Pos8
Proteinase K	Hard-shell thin-wall 96-well skirted PCR plates	Pos17

Name	Consumables	Position
cfDNA	Hard-shell thin-wall 96-well skirted PCR plates	Pos12

15. Tap **Start** , select the sample type in the pop-up window, and tap **Continue** . The extraction workflow starts. It takes about 80 minutes.
16. After the workflow is completed, take out the product (50 µL) at Pos12 and store it at -20 ℃ or below.
17. Dispose of the used deep-well plates, PCR plates and waste bag, and transfer them to the designated waste area.

    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*.

4.5 Extracting the nucleic acids with MGISP-100RS

4.5.1 Preparing the consumables

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

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	2 Boxes
1.3 mL U-bottom deep-well plate	MGI	1000004644	1 Plate
Break-away 8 Strips PCR Tubes and Caps	MGI	100-000016-00	4 Strips

4.5.2 Preparing the samples

MGISP-100RS can process 1-16 samples at one time. Take out the samples and thaw them at room temperature, centrifuge and mix them thoroughly.

4.5.3 Preparing the reagents

Perform the following steps:

1. Add an approximate absolute ethanol into Buffer W2 according to the label on Buffer W2 bottle.

2. Take out one 1.3 mL U-bottom deep-well plate (MGI, 1000004644), four Break-away 8 Strips PCR Tubes and Caps (MGI, 100-000016-00), mark them, and add the corresponding reagents according to the volumes listed in the following table.

Marked name	Consumable	Volume/well
Proteinase K	Columns 1 and 2 of deep-well plate	15 μL
Sample		300 μL
Mini Buffer LYS		290 μL
Mini Wash Buffer 1-1	Columns 5 and 6 of deep-well plate	770 μL
Mini Wash Buffer 1-2	Columns 7 and 8 of deep-well plate	770 μL
Buffer W2	Columns 9 and 10 of deep-well plate	770 μL
Isopropyl alcohol	Column 11 of deep-well plate	370 μL
Magnetic Beads	Column 1 of PCR Tubes	35 μL
TE Buffer	Column 2 of PCR Tubes	110 μL

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

4.5.4 Starting extraction

Perform the following steps:

1. Double-click the control software icon on the desktop. The Login interface is displayed. Select **Real** and click **Create** .
2. Click **Initialize** in the main interface.
   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.
3. Click the menu button and select **Run Wizard** .
4. In the Run Wizard interface, click the **Solution** list and select **JB-A06-121 Nucleic acid extraction Kit_RV2.0_SV1.0** . Click the **Script** list and select **JB- A06-121 Nucleic acid extraction Kit_RV2.0_SV1.0** .

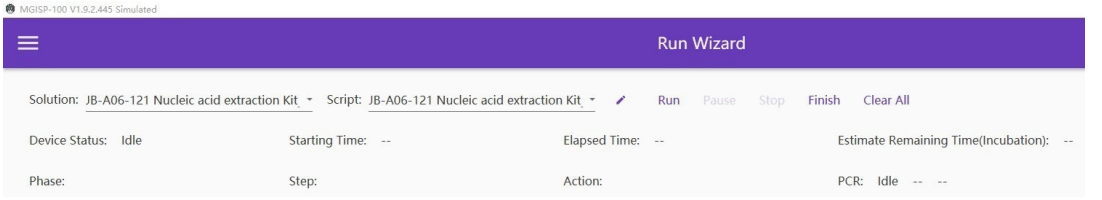

Figure 3 Run Wizard interface

5. The operation deck arrangement is displayed as shown in the following table. Follow the table below to place the samples, reagents, and consumables, confirm the placement, and close the door.

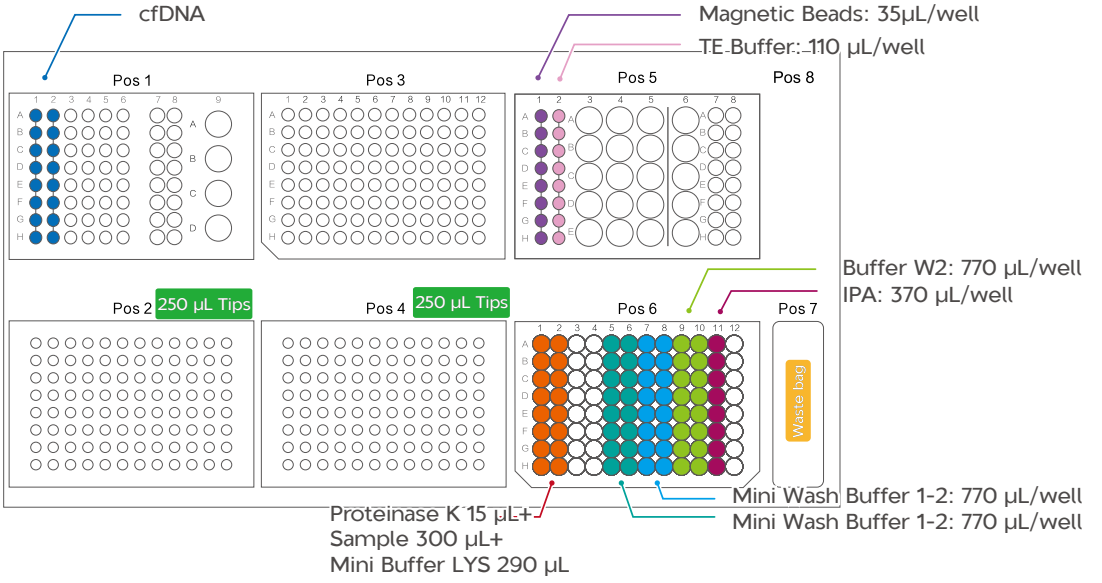

Figure 4 Operation deck arrangement

Table 6 Deck positions of the sample, reagent, and consumables

Name	Consumable	Position
250 μL automated filter tips	/	Pos2, Pos4
Magnetic Beads	Break-away 8 Strips PCR Tubes	Pos5-1
TE Buffer	Break-away 8 Strips PCR Tubes	Pos5-2
cfDNA	Break-away 8 Strips PCR Tubes	Pos1-1, Pos1-2
Reagents and samples	1.3 mL U-bottom deep-well plate	Pos6

6. Click **Run** . A window is displayed. Select 1-16 from the **Please enter the sample number list** and click **Continue** .
7. The workflow takes about 100 minutes. After the workflow is completed, take out the product (50 μL) at Pos1-1 and Pos1-2 and store it at -20 ℃ or below.

8. Dispose of the used deep-well plates, PCR plates, and waste bag to the designated waste area. Perform a post-clean according to *MGISP-100&MGISP-960 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

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