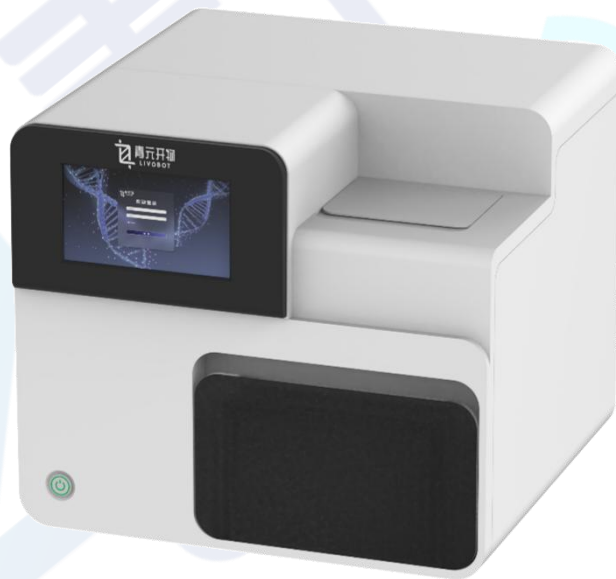


EoSonics[®] FRAG8

Focused Ultrasonicator

User Manual

Version 1.1



For Users of LivoBot Products Only

For Research only

2024.2



End User License Agreement

User Notice:

Please read this end user license agreement (hereinafter referred to as the "Agreement") carefully. This Agreement is entered into by and between you (including natural person, corporation, organization or any other entity) and LivoBot Co., Ltd. (hereinafter referred to as "LivoBot") as a legal binding agreement regarding the EoSonics® FRAG8 focused ultrasonicator (hereinafter referred to as the "Instrument").

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Under normal use, this Software carrier is guaranteed free of defects in material or workmanship for a period of ninety (90) days from the date of sale.

- In case of defects upon verification, the sole responsibility of LivoBot is to return or replace its software carrier, which is also your sole compensation.
- THE FOREGOING WARRANTY WILL BE VOID IF CARRIER DEFECTS OR MEDIA DAMAGES ARE CAUSED BY MISUSE, ACCIDENT, ABUSE, MODIFICATION OR MISAPPLICATION.

Excluding the aforementioned conditions, defects in this Software System are not entitled to any other form of after-sales warranty.

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UNDER ANY CIRCUMSTANCE, THE MAX LIABILITY OF LivoBot BY ANY TERM OF THIS AGREEMENT IS LIMITED TO THE MONETARY AMOUNT YOU PAID FOR THE SOFTWARE SYSTEM.

Termination of License:

LivoBot may terminate this license at any time if any of the terms and conditions of this Agreement is violated. Upon termination of the license, you must immediately destroy all copies of the System and documents, or return them to LivoBot.

Applicable Law and Jurisdiction:

This software product is protected by the laws of the People's Republic of China and the effective international intellectual property treaties concluded or acceded to by China.

This Agreement shall be governed by the laws of the People's Republic of China.

Any dispute arising from this Agreement shall be negotiated by both parties under the principles of friendship and good faith. If negotiation fails, Beijing Arbitration Commission shall intervene for arbitration.

LivoBot reserves the right of final interpretation of this Agreement.

If you have any questions concerning this Agreement, please contact LivoBot Co., Ltd.

Contact information:

Address: South Part, 12th Floor, Building 6, PKU Healthcare Innovation Valley, Life Science Park, Changping District, Beijing

Tel.: +86-18311378180

E-mail: qingyuankaiwu@livobot.com



Preface Information

Before-Use Instructions:

This manual applies to the use of EoSonics® FRAG8 focused ultrasonicator. Before you operate this instrument, please read the manual carefully to gather necessary information about the instrument. Please operate in strict accordance with the instructions in the manual. If in any case there is an operation question, please contact us.

Definitions

Keypads, buttons, menu items, Technical terms, windows, dialog boxes, sub-menus and form controls mentioned in the text of this manual are marked with []; . For example:

Click [Run] to start the experiment.

Get Support

If you encounter any problems and need help during installation and operation of the EoSonics® FRAG8 focused ultrasonicator, please contact technical support of LivoBot Co., Ltd. in Beijing. The contact information is as follows:

Address: South Part, 12th Floor, Building 6, PKU Healthcare Innovation Valley, Life Science Park, Changping District, Beijing

Tel.: +86-18311378180

E-mail: qingyuankaiwu@livobot.com

When you call, please prepare the following information:

- Serial number of the EoSonics® FRAG8 system (on the back panel of the instrument)

- Version of the Software

- Description of the problem

- Methods and operation steps you use

- Your contact information such as telephone number, email address and working address

Product Information

Product name: Focused ultrasonicator

Model: EoSonics® FRAG8

Date of manufacture: See product label

Service life: 5 years

Date of instructions prepared: February 20, 2024

Document Version: V1.1

Chapter 1 Product Description

1.1 Product Introduction

EoSonic[®] FRAG8 focused ultrasonicator (EoSonic[®] FRAG8 system for short) adopts ACU[™] (adaptive cylindrical ultrasonic) focused acoustics technology to process nucleic acids, chromatin or cultured cells. The size of the DNA fragmentation produced by the system is controllable by fine-tuning the acoustic parameters.

The EoSonic[®] FRAG8 system can flexibly accommodate 1 to 8 samples per run using standard commercial consumables such as 8-tube strips, thus minimizing the cost of consumables when running at different throughputs. As a hands-free automated solution, the EoSonic[®] FRAG8 system eliminates the need for manual loading of samples into specialized vials or tubes during operation, such that the loaded samples can be directly fragmented. In contrast to traditional enzyme-based fragmentation, physical ultrasonic shearing offers the advantages of no breaking preference to DNA bases and relatively narrow size distribution of DNA fragments, making it the preferred option in fragmenting DNA samples.

1.2 Instrument Composition

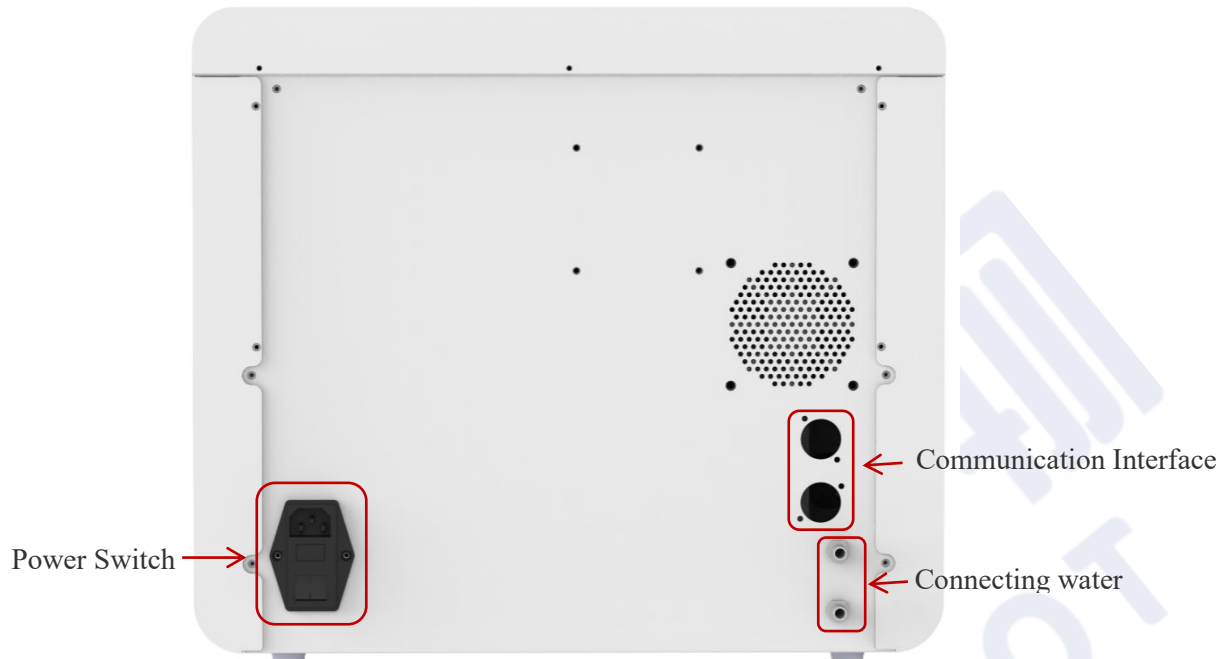
EoSonic[®] FRAG8 system is mainly made up by a host computer and the operating software.

1.2.1 Structural Diagram of EoSonic[®] FRAG8 System

(1) Front view



(2) Rear view



(3) Side view



1.3 Intended Usage

This instrument is designed to break the extracted long-chain nucleic acid and chromatin, and the size of the DNA fragmentation produced by it is controllable by fine-tuning the acoustic parameters.

1.4 Scope of Application

This system is designed for testing facilities, such as laboratories in academic institutions, and Centers for Disease Control and Prevention and clinical labs, to break or shear nucleic acids and chromatin samples.

1.5 Operating Power Supply

- (1) Supply voltage: AC220–240 V, frequency 50 Hz;
- (2) Input power: 800 VA.

1.6 Requirements for Transportation, Storage and Operating Environment

Environment

1.6.1 Requirements for Transportation and Storage Environment

- (1) Instrument transportation requirements: The focused ultrasonicator is 417 mm long, 438 mm wide, 385 mm high, and weighs about 25 kg. It can be moved manually with no help of tools and equipment. Handle with care during lifting and moving. The bottom of the instrument should be firmly held by hands to prevent accidental drop, which may cause personal injury and instrument damage. If long-distance transportation is required, the instrument should be repacked. Avoid applying pressure or placing heavy objects on the instrument to prevent any damage during transportation.
- (2) The packaged product should be stored horizontally in a well-ventilated room with the temperature controlled at -20°C – 50°C , the relative humidity $\leq 80\%$, and without condensation or corrosive gas.

1.6.2 Requirements for Operating Environment

- 1) Scenario: Indoor, at an altitude below 2000 m;
- 2) Ambient temperature: 10°C – 30°C ;
- 3) Relative humidity: $\leq 80\%$, no condensation;
- 4) MAINS supply voltage fluctuations up to $\pm 10\%$ of the nominal voltage;
- 5) Pollution degree: PD 2;
- 6) Not for wet condition.

1.7 List of Accessories

All accessories of the instrument are documented in the table below. If you find any missing items after unpacking, please contact our after-sales service.

Table 1-1 List of main accessories

Sequence No.	Name	Model	Quantity	Units
1.	Focused Ultrasonicator	EoSonics® FRAG8	1	Set
2.	Water Circulating Cooling Systems	Ref-8	1	Set
3.	Power cord	-	1	Piece
4.	Communication line	-	1	Piece
5.	Clamp	-	2	-
6.	Fuse	T5AL250V	2	-
7.	User manual	-	1	-
8.	Certificate	-	1	-
9.	Warranty card	-	1	-

Chapter 2 Precautions

2.1. Safety Precautions

Please read the "Safety Precautions" in the manual carefully before use. All important safety-related contents here are intended to guide users to operate the instrument safely and correctly and to prevent users and the instrument from being harmed or damaged. Be sure to comply with all guidelines and use the instrument properly.




2.1.1. Users of the instrument

The instrument should only be operated by professionals who have undergone systematic training. They should possess a comprehensive understanding of proper operation procedures as well as safety protocols.

2.1.2 Potential safety hazards and usage restrictions

- **Safety tips marked in this manual**

The instrument has no user-repairable components. Do not disassemble it in case of malfunction. Please follow the safety precautions in this manual:

	<p>Warning:</p> <p>Failure to adhere to safety precautions regarding warnings or operations may cause serious harm to users or others.</p> <p>This symbol indicates the need for the user to consult the instructions for use to determine the nature of the potential hazard and the necessary prevention measures.</p>
	<p>Caution:</p> <p>This symbol indicates the possibility of instrument damage, loss of data, or poor performance of the system if the instructions are not followed.</p>
	<p>Warning:</p> <p>Beware of mechanical injury. Do not place your fingers here while this part is moving.</p>

- **Protective guarding door and enclosure of the instrument**

The protectively designed guarding doors and enclosures of the instrument prevent you from exposure to bright LED light, high voltage, and moving parts. During normal operation, you will not come into contact with the internal structure of the EoSonics® FRAG8 system.



Warning:

When the machine is on the run, do not open the protective guarding door and enclosure.

Opening the guarding door of the instrument may cause injury to you or others; Opening the instrument enclosure may cause damage to the internal parts of the instrument, as well as the risk of high voltage and damage to the internal circuit.

● Moving Parts

The moving parts are inside the experimental chamber of the instrument. During normal experiments, you will not come into contact with the internal moving parts.

When the instrument is placed on the table, the distance from the front panel of the instrument to the edge of the table should be at least 2 cm.



Warning:

Beware of mechanical injury. Do not place your fingers here while this part is moving.

● Biohazard

The EoSonic[®] FRAG8 system works on processed nucleic acid or chromatin samples, which are easily contaminated and degraded. When using the instrument to process samples, it is imperative to wear protective gloves and medical masks.



Warning:

Samples processed by the EoSonic[®] FRAG8 system must be handled in strict accordance with biological operation specifications, applicable laws and regulations, as well as the guidelines provided by the local health authority.










2.1.3 Precautions

- (1) Please adhere to the instructions provided in the manual when using the instrument to ensure that the protection provided remains effective and the instrument is not damaged.
- (2) To prevent personal injury or death caused by electric current leakage, please ensure that the equipment is used under the condition that the socket ground wire is effectively grounded;
- (3) The instrument should only be operated by professionals who have undergone systematic training. They should possess a comprehensive understanding of proper operation procedures as well as safety protocols.

2.2 Handling Protocol during Emergency

- (1) If any abnormality arises during the operation of the instrument, disconnect the power immediately and stop using the instrument;
- (2) In case of instrument failure, notify professional technicians for repair. To avoid danger, never disassemble, repair or modify the equipment by yourself.

2.3 Description of Labeling

Label	Label Description
	Indicates that caution is necessary.
	Warning: Indicates that exposure to high pressure may cause serious injury.
	Warning: Beware of mechanical injury. Do not place your fingers here while this part is moving.
	Warning: Indicates that operation and handling should be carried out in accordance with biological operating procedures, relevant laws and regulations, and the guidelines of the local health authority.
	Warning: The grounding must be correct and firm. Incorrect grounding may lead to the risk of electric shock.
	Indicates that the transport package contains fragile items and can be broken or damaged if not handled carefully.
	Indicates that the transport package shall be vertically upward during transport
	Indicates that the transport package needs to be protected from rain.
	Indicates the layer location of the packing code for the transport package.

Chapter 3 Installation and Operation

3.1 Checking before Installation

3.1.1 Inspect before Unpacking

The instrument undergoes rigorous inspection by our team of professionals before packaging and shipment and is then shipped to the installation site by a designated shipping company.

After receiving the package, users should carefully inspect the outer packaging for the following damages before unpacking:

- (1) Deformation;
- (2) Obvious impact marks;
- (3) Signs of unpacking.

Once the above damage is found, please do not unpack the package, and immediately contact our after-sales service.

3.1.2 Unpacking and Checking

After unpacking, please check all contents by the packing list. If any items are found missing or damaged, please contact our after-sales service.

After the packing list check, take the instrument out of the packing case and place it on a horizontal desktop.

3.2 Installation

- (1) The instrument should be placed on a desktop in a well-ventilated room with no interference from flammable, explosive, corrosive gases and strong magnetic field.
- (2) Do not use this instrument in places exposed to direct sunlight. Keep away from heat sources such as heaters.
- (3) Please keep the installation table clean and tidy to prevent debris from affecting the use of the instrument.
- (4) The instrument should be placed on a stable desktop, and the distance around the instrument from the wall should be no less than 10 cm.
- (5) Connect one end of the power cord to the power socket on the back board of the instrument and the other end to the mains outlet using the power cord with the factory standard configuration.

3.3 Precautions for Use

- (1) Please carefully read the user manual before using the instrument;
- (2) Please ensure that the installation and use conditions of the instrument meet the requirements;
- (3) Do not place sundries on the instrument, and keep the operating environment clean;
- (4) During operation, handle with care and avoid excessive force to cause damage to the instrument;
- (5) Before experiment, check that the orifice plate is placed in accurate position with its notch facing outward. If unchecked, it may cause instrument abnormality and affect experiment results;
- (6) During experiment, a small amount of water mist may volatilize in the experimental cabin of the instrument. Experimenters and operators must take necessary protective measures,

such as wearing work clothes, isolation gowns, rubber shoes, masks, gloves, and work caps; Wear goggles if feasible;

- (7) Disposable consumables, reagent and sample residues, disposable protective gloves and masks, etc. used in the experiment should be properly disposed in accordance with the biochemical laboratory operating procedures. Laboratory wastes should be classified and placed into non-biohazardous or biohazardous garbage bins fitted with qualified disposable bags, and disposed of by harmless treatment afterward;
- (8) Upon arrival, carefully move the instrument to the destination area in the laboratory while avoiding collision to damage the instrument.

3.4 Quick Starting Guide

(1) Power Connection

Connect one end of the power cord to the power port at the rear of the instrument, and connect the other end to a power socket.

(2) Starting Up

Turn on the power switch and start button on the instrument. The system software will start automatically.

Chapter 4 Experimental Operation

4.1 Starting up the System

- (1) Power up the instrument;
- (2) The software runs automatically and enters the system.

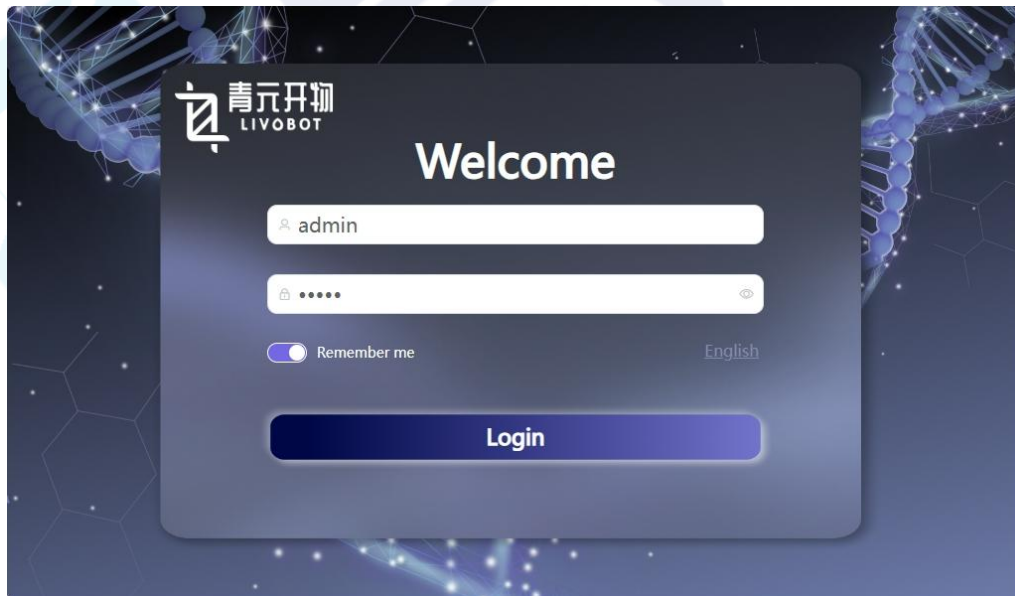
4.2 Overview

This section briefly introduces the experimental operations and explains the sequence steps in software operations. It is recommended that you read this section thoroughly. The software version used in this chapter is V1.3.

Keypads, buttons, and menu items mentioned in this section are marked with **【】**; Technical terms, windows, dialog boxes, sub-menus and form controls are marked with **[]**. For example: Click **【Run】** to start the experiment.

4.3 Operation Sequence

- (1) Start up the instrument to enter the login interface. Key in the account password and click "Login"



Login Interface

- (2) Cooler Operation

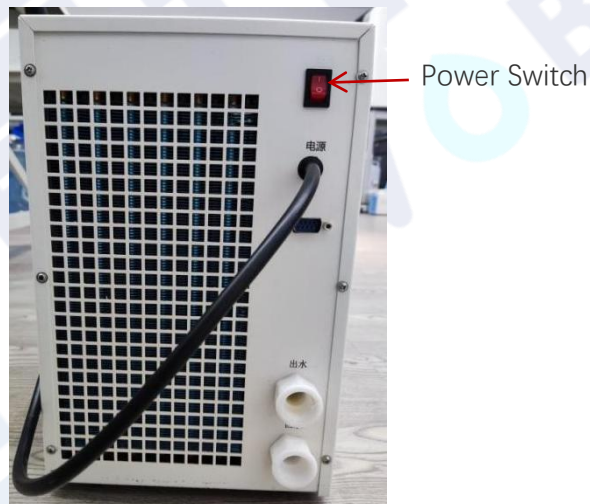
Pull out the water tank. You don't need to pull the tank all the way out but enough working space for adding water. Use experimental water of grade 3 or higher. Add water into the tank until water level reaches the zero mark.



Zero Mark of Tank

Slowly push the tank back into the instrument Do not push hard to avoid violent fluctuations in water surface, causing water spill out of the tank.

Turn on the power switch of the cooler to start up the cooling system.

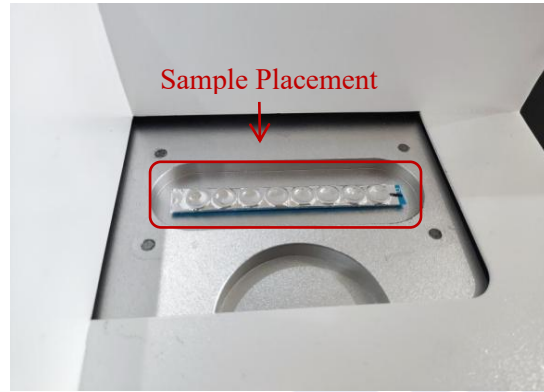


Note: It is strictly prohibited to turn on the cooling system when there is no or little water.

(3) Preparation of Sample

First add 2 small stirring rods into each reaction pool, then place samples in each reaction pool. Finally seal the membrane on top of the reaction pool, shake, and centrifuge. The shaking and centrifugation steps can be omitted if the sample does not need to be diluted, or if the sample, before being added to the reaction pool, has been diluted outside the PCR plate.

(4) Sample Placement

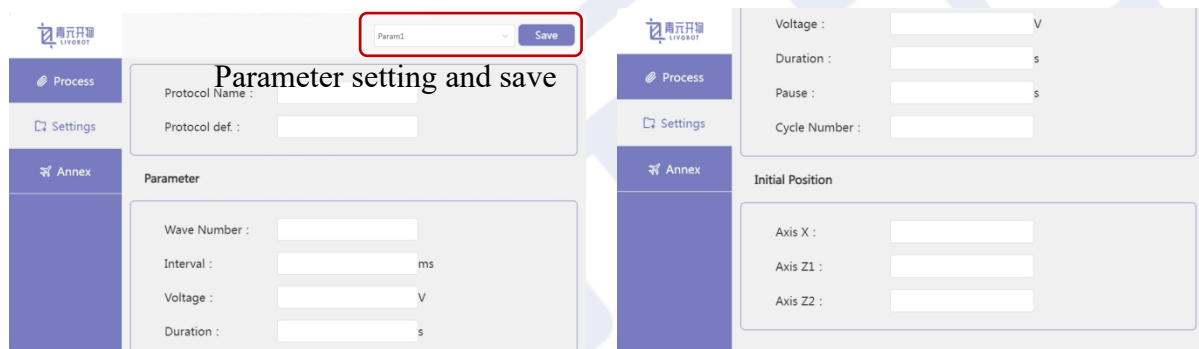


Open the right flip cover of EoSonics® FRAG8, as shown in the figure above.

Remove the orifice plate cover, put in the sample-loaded orifice plate, then place back the orifice plate cover and close the flip cover.

(5) Parameter Settings

Parameter setting interface is shown below:



【Excitation Cycles】 : Number of periodic ultrasound pulses, range 1-1500

【Excitation Frequency】 : Frequency of ultrasound pulse (Hz), run by the default value.

【Excitation Interval】 : Interval time between individual pulses, 1-100 ms

【Excitation Voltage】 : Average voltage, 1-90 volt

【Treatment Time】 : The time duration for ultrasound to act on the sample, ranging from 1-1200 s.

【Interval Time】 : Intermittent time between two sequential ultrasound treatments, range 1-1200 s

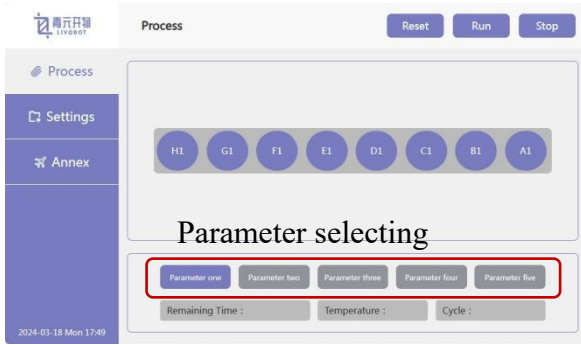
【Cycle Numbers】 : Number of repeating the ultrasound cycles, 1-100

After completing parameter settings, click **【Save】** and the message **【Save Successfully】** will pop up, indicating that parameter setting is completed.

(6) Setup the Running Process

Select the test samples in a row (designated by different letters, shown as, e.g., H1, G1, F1, ..., A1 in the center area of the figure) from the main screen. When selected, the designated letter pad will turn blue. Re-clicking the selected sample again aborts the selection, and the LCD keypad turns gray.

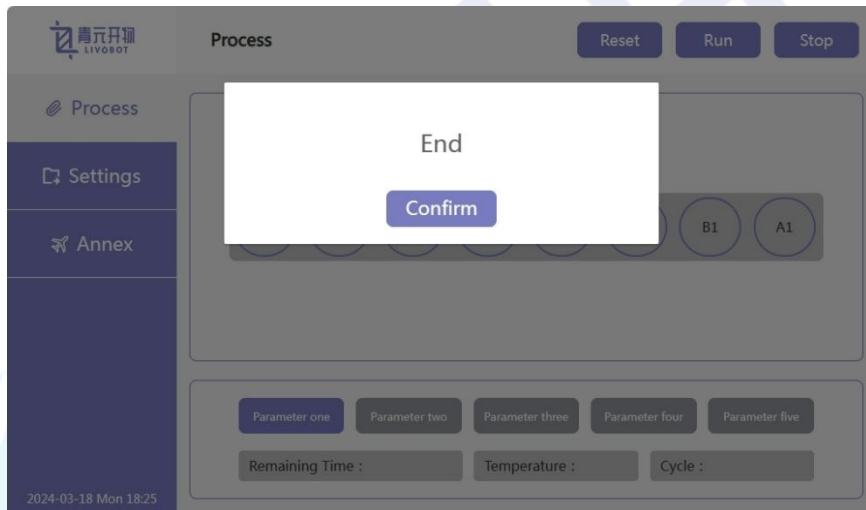
Click **【Run】** (shown in the upper-right corner of the main screen for the "Process" module) and the message **【Start Running】** will pop up in the main screen, as shown in figure, indicating that the selected process has started to run normally. A timing countdown will also be displayed in the "Remaining Time:" panel at the bottom of the main screen.



Note: It is strictly prohibited to run the instrument with the flip cover open to avoid harm to human body caused by ultrasonic wave.

(7) End of run

When the operation is over, an end prompt box will pop up. Click **【Confirm】**, as shown below.



Chapter 5 Maintenance

5.1 Overview

The EoSonics® FRAG8 system is a precision instrument that requires routine maintenance to ensure work quality. New versions and new functions of its control & analysis software will also be updated and released.

5.2 Key to System Hardware Maintenance

- (1) The enclosure of the instrument shall be cleaned with a soft cloth, and no liquid detergent shall be used;
- (2) Do not switch the instrument on and off frequently. The time interval between switching on and off should exceed 30 seconds;
- (3) Do not plug or unplug the communication cable frequently;
- (4) When testing the connection of the communication line of the instrument, the time interval between inserting/removing the communication line into/out of the communication port should not be less than 5 seconds;
- (5) Do not place the instrument on an uneven desktop;
- (6) Do not place objects on the instrument;
- (7) Install and use the instrument according to the operation methods provided in this manual;
- (8) When the instrument needs to be moved, pay attention to avoid shaking.
- (9) Do not replace detachable MAINS, supply cords by inadequately RATED cords.

5.3 Instrument Maintenance

5.3.1 Routine Maintenance

- (1) The water added to the tank is at least pure water level, to ensure that the sound wave conduction is not affected;
- (2) At the end of the experiment, clean the orifice-plate adapter rack and flip cover with 75% ethanol;
- (3) The surface of the instrument enclosure shall be cleaned regularly with soft cloth, and avoid the use of strong alkali, concentrated alcohol and organic solvent solution;
- (4) Keep the environment in the experimental chamber dry and free of water stains;
- (5) Please ensure ventilation around the instrument when in use;
- (6) The water in the tank is changed every week, and the tank is cleaned every two weeks.

5.3.2 Periodic Maintenance

- (1) Change the water every week to prevent impurities in the water from affecting the sound wave conduction;
- (2) Clean the tank every two weeks to prevent microbial breeding;
- (3) Check the pipes and components of the equipment once every six months to see whether

- they are loose and whether water leaks;
- (4) Calibrate the transducer every six months;
 - (5) Calibrated the position of the motion module every six months;
 - (6) The temperature control function is tested every six months to ensure the cooling effect during the operation of the instrument;
 - (7) The instrument should be routinely inspected and maintained every year, or maintained by maintenance engineers according to the maintenance plan or customer requests.

5.4 Process for Reuse Treatment

The instrument can be reused. During use, routine maintenance should be performed.

5.5 Disposal after expiration of instrument life

The instrument is designed for a service life of 5 years. After the service life expiration, the instrument should be disposed of according to relevant procedures of medical institutions and the relevant requirements of local environmental protection provisions.

Note: The instrument requires regular maintenance to ensure working quality.

Chapter 6 Troubleshooting

6.1 Failures and Solutions

- Do not disassemble or open the device at will to avoid the risk of electric shock.

Failure Description	Solutions
No display on the screen when the instrument is turned on	<ol style="list-style-type: none"> (1) Check whether the power socket is plugged; (2) Check whether the power cord is firmly connected; (3) Check whether the power switch is turned on; (4) Check whether the fuse of the instrument is blown. If yes, replace the fuse.
Abnormal noises	<ol style="list-style-type: none"> (1) Check whether the instrument is placed stably on the desktop; (2) Check whether each moving part is in its correct position.
The motion axis cannot operate normally	<ol style="list-style-type: none"> (1) Check whether any moving part is stuck; (2) If there are still abnormalities, please contact the supplier.
System crash or loss of control	<ol style="list-style-type: none"> (1) If the system crashes due to improper operation, cut off the power and restart the instrument; (2) If there are still abnormalities, please contact the supplier.

Conditions requiring contact with suppliers	<p>Note: On the occurrence of any of the following events, cut off the power immediately and contact the supplier or the company, so that it can be handled by professionally trained maintenance personnel:</p> <ol style="list-style-type: none">(1) Liquid spilled into the instrument;(2) Rain or water exposure;(3) Any abnormal sound or odor emitted from the instrument;(4) Damaged enclosure due to falling of the instrument;(5) Significant changes in instrument function.
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