

For research use only

L8TM

USER MANUAL

A single microplate system developed for high performance sample preparation using adaptive focused acoustic energy.

Model Number: L8



UNIVERSAL PRECAUTIONS

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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Not for use in diagnostic procedures

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Warnings

For safety of operating personnel:

1. Make sure the equipment is properly grounded. **DO NOT** operate if it is not properly grounded.
2. The unit is equipped with a power plug appropriate for the destination country. **DO NOT**, under any circumstances, remove the grounding prong from the power cord.
3. **DO NOT** attempt to operate the equipment with the Front cover open, or without a water bath; the acoustic system will not work. Ultrasonic and pinch hazards exist in the treatment compartment while the instrument is processing plates. If there is any indication that the Safety System is not functioning properly, **DO NOT** operate the equipment and contact Covaris immediately.
4. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

To prevent damage to the equipment:

1. The instruments are designed to operate in ambient laboratory conditions e.g., 15 to 32°C (60 to 90°F). **DO NOT** operate the instrument in a cold room environment; the system is designed to operate with a water bath and re-circulating heater/chiller apparatus to control sample temperature.
2. **NEVER** run a method without a water bath; this could damage the transducer. The instrument is equipped with a water level sensor to protect the transducer, fountain pump and degassing pump. The system will not allow the degassing and fountain pumps to operate for longer than 10 seconds or the acoustic wave treatment to start unless an adequate volume of water is detected.
3. Distilled or deionized water should be used to fill the water bath.
4. Empty the water bath and wipe it dry **EVERY** day with a lint-free cloth. **DO NOT** leave water in the tank for an extended period of time as there is no water filtration or water cleaning system with the apparatus.
5. The Acoustic Assembly should be left in the up or Service position when not in use.
6. Establish a standard of operation and periodically test equipment, as described in Maintenance section (see Section 0) of this manual.
7. **DO NOT** load third party software onto the computer controlling the system without consulting with Covaris.

Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery by an authorized representative. Any component which proves defective during the stated period will be repaired free of charge or replaced at the sole discretion of Covaris, F.O.B., Woburn, Massachusetts, U.S.A. provided the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement with telephone support from Covaris personnel. If Covaris personnel are required to perform on-site repair, all travel related costs are paid by the customer. A limited warranty as specified may apply to certain components of the equipment.

Warranty Exceptions

This warranty is void if failure of the software or hardware has resulted from accidents, abuse, improper maintenance, or repair, or misapplication by the customer. It is also void if damage is caused by any unauthorized attachments or if modifications are made to the equipment. Removing or tampering with the Safety Enclosure will void the warranty, and the customer will assume all liabilities.

This warranty is limited to the original purchaser and is not transferable.

The software will perform according to the accompanying written materials and the medium on which the software is delivered is free of defects in materials under normal use and service. The warranty is void if damage has resulted from third party software not intended for use with the system.

The high power focused transducer is designed to give maximal mechanical energy output in water. Permanent damage to the transducer and electronic circuits could result if the transducer is operated without water. Operation of the system without water in the water bath voids the warranty.

CONTACT COVARIS, INC. SHOULD YOU HAVE ANY QUESTIONS CONCERNING EQUIPMENT

Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the service and support necessary so that the customer can operate the equipment successfully. Extended warranties are available at the end of the original 12 month warranty period.

Installation and Training – Setup and installation of the equipment and operator training can be purchased at time of initial acquisition of the equipment. Both will be performed by a qualified Covaris service representative. One half day will be scheduled to perform the installation and on-site training.

- The operators' training will include valuable hands-on time with the equipment.
- Preventative maintenance and troubleshooting tips will also be covered.

Services included with the original purchase of the system are:

Technical Support – On-going assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone (+1 781 932 3959) during the hours of 9:00am to 5:00pm, Monday through Friday,
 - United States, Eastern Standard Time (EST)
 - Greenwich Mean Time (GMT) - 05:00
- E-mail queries to techsupport@covarisinc.com

Parts Replacement – Replacement of parts (excluding consumables) from normal operation of equipment are provided on a priority basis. All labor and shipping charges are included. Failure due to accident, abuse, or improper operation is not covered.

TABLE OF CONTENTS

1.0	INTRODUCTION	7
1.1	Overview of the Manual	7
1.2	Purpose of the Equipment.....	7
2.0	UNPACKING AND INSTALLATION	7
2.1	Unpacking and Handling	7
2.2	Placement of Equipment	8
2.3	Power Requirements.....	8
2.4	Connections.....	8
2.4.1	Chiller Connection.....	8
2.4.2	Power and Communication	8
3.0	SYSTEM DESCRIPTION	9
3.1	Main Components	9
3.2	General Overview	9
3.3	Principle of Operation	10
3.4	Treatment System	11
3.4.1	Acoustic Assembly	11
3.4.2	Acoustic Transducer.....	11
3.4.3	Temperature Sensors and Temperature Control	11
3.4.4	Water Bath and Degassing System	12
3.4.5	Fountain Pump	12
3.4.6	Computer.....	12
3.5	Safety System	13
	Clear Cover	13
	Plate Handler Drawer Front.....	14
3.6	Application Software.....	14
4.0	SYSTEM OPERATION	14
4.1	Start-Up Procedures	14
4.1.1	Water Bath Configuration	14
4.1.2	Water Bath.....	15
4.1.3	Powering Up the System	15
4.1.4	Interior Light	16
4.1.5	Initialization.....	16
4.2	Operating Procedure.....	17
4.2.1	Main Panel Screen.....	17
4.2.2	Method Editor Screen	22
4.2.3	Degassing	24
4.2.4	Loading the microplate	25
4.2.5	Selecting the Method	25
4.2.6	Processing the Plate	25
4.2.7	Processing Complete.....	27
4.3	Shutdown Procedure	27
4.3.1	To purge the degassing and fountain pumps:	28
4.3.2	To shut down the instrument:	29
4.3.3	To shut down the laptop.....	29
5.0	SYSTEM SPECIFICATIONS	30
6.0	MAINTENANCE	30
6.1	Air intake	31
6.2	Water Tank	31
6.2.1	Recommendations for Daily Maintenance.....	31
6.2.2	Recommendations for Monthly Maintenance.....	31
6.3	Transducer	31
6.4	Safety System	31
6.5	Pump, Hoses, and Cables	32
6.6	Relocation of the System	32
6.7	Cleaning the System	32
7.0	TROUBLESHOOTING	33

APPENDIX A.....	34
APPENDIX B.....	35
APPENDIX C.....	36

LIST OF FIGURES

Figure 1 The Covaris L8 System.....	9
Figure 2 Front view and main components.....	10
Figure 3 Disabling the Tapping feature.....	13
Figure 4 Front panel indicator lights.....	15
Figure 5 System start-up dialog with initialization options.....	17
Figure 6 Main Panel of software application.....	18
Figure 7 Water bath temperature warning.....	19
Figure 8 Method Folder selection with SELECT CUR DIR button.....	20
Figure 9 Method Editor.....	22
Figure 10 Plate Folder selection with select CURRENT FOLDER button.....	24
Figure 11 Retract Plate reminder dialog.....	26
Figure 12 System Not Fully Degassed dialog and options.....	26
Figure 13 Interlock fault at start of method.....	27
Figure 14 Interlock fault during method in progress.....	27
Figure 15 Plate processing complete dialog.....	27
Figure 16 Water level warning dialog for purging the degassing pump.....	28

1.0 INTRODUCTION

1.1 Overview of the Manual

This manual contains operation and service instructions for the Covaris L8 instrument. It contains background information essential to the proper use and care of this equipment.

Should any unforeseen problems occur with the normal operation of the equipment, contact Covaris Technical Support immediately.

The following definitions apply in this manual:

NOTE: Inconvenience if disregarded.

CAUTION: Equipment damage may occur.

WARNING: Personal injury may occur.

There is also a PDF version of this manual on the system computer and/or software installation CD. Open the manual by double clicking the manual icon on the desktop.

1.2 Purpose of the Equipment

The L-series instruments are intended specifically for multiple sample preparation and compound mixing. The system is designed to work with a variety of microplates and well volumes from 96, to 384 and 1536 formats. (Contact Technical Support for more information.)

The instruments are not intended for use in diagnostic procedures; they are designed for research use only.

2.0 UNPACKING AND INSTALLATION

2.1 Unpacking and Handling

The Covaris system is shipped in one box containing the instrument, the instrument supplies, and an optionally supplied computer. Remove all wrappings, taking care not to inadvertently discard any parts, accessories, or documents.

Normal precautions in unpacking and reasonable care in handling should be exercised to avoid possible damage to the unit. Visually inspect all external controls, indicators, and surfaces to detect any damage that may have occurred during shipment. Remove shipping foam from around the acoustic assembly.

Included in the shipping box:

- L8 instrument
- Two hoses with connectors for recirculating chiller/heater
- USB cable
- Power cord for L8
- Software CD (SonoLAB and drivers) and User Manual
- Water tank
- Nozzle replacement kit (p/n 500124)
- Notebook computer (optional)

The shipping company is responsible for damage to the equipment during shipment. If damage has occurred, notify the shipping company immediately to establish proper basis for a claim.

If the recirculating chiller/heater was ordered from Covaris it is shipped in a separate box.

NOTE: In case of damage, do not discard packing material until Covaris has inspected it for cause of damage.

2.2 Placement of Equipment

The area required for the instrument (see 0) is approximately 15" wide by 17" deep by 14" high (38cm x 43cm x 36cm). Additional clearance space of approximately 10" (25cm) in front of the unit and 15" (38cm) above the unit is required for access to the water tank. Also, additional space is required for a computer and a user-supplied recirculating chiller/heater.

To accommodate the input and exhaust of internal fans, place the instrument so that there is a minimum of 1" (3 cm) between the instrument, on all sides, and any wall or obstruction.

2.3 Power Requirements

The instrument draws a maximum of 300 VA. The Covaris L8 instrument is configured with a "universal" power supply. Refer to label on the back of instrument for specific power requirements.

WARNING: To prevent the possibility of electrical shock, always plug the system into a grounded power source.

2.4 Connections

2.4.1 Chiller Connection

A recirculating chiller (required) should be connected to the treatment system using the hoses supplied. The chiller inlet is connected to the fitting labeled "IN" and the chiller outlet is connected to the fitting labeled "OUT" located on the back of the instrument.

1. Slide the ends of the hoses over the inlet and outlet nozzles at the rear of the chiller.
2. Tighten the hose clamps with a screw driver.

2.4.2 Power and Communication

Connect the female end of the included power cord into the back of the instrument and plug the male end of the power cord into a grounded power source.

Connect the computer to the instrument using the USB cable supplied with the unit, from the USB connector on the back of the instrument to any USB port on the computer

3.0 SYSTEM DESCRIPTION

3.1 Main Components

The main components of the L8 instrument include:

- Acoustic Assembly – transducer, motion control and water fountain nozzle
- Plate handler and motion controlled loading mechanism
- Temperature sensing device for water bath with cooling loop
- Degassing system with water level sensor
- Fountain pump
- Safety Interlock System, including clear door and plate handler

External components include a computer (optionally supplied by Covaris) and a recirculating chiller/heater (not shown) to be supplied by user.

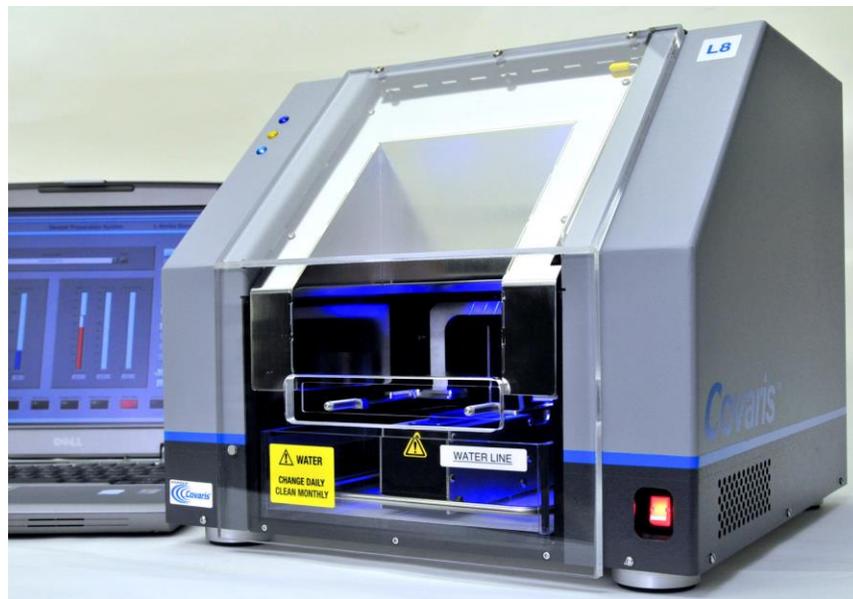


Figure 1 The Covaris L8 System

3.2 General Overview

The *Covaris L8* is a precision instrument for controlled sample preparation and mixing. The system is comprised of the Treatment System which delivers the energy to the sample, the Safety System which protects users from accidental contact with the acoustic energy, and the Computer with the Application Software, which provides the user interface to the system.

This instrument is a very powerful, high-energy system. It is designed to disrupt and homogenize biological tissue, extract biomolecules, and accelerate chemical dissolution. 0 depicts the Acoustic Assembly, Water Sensor, Chiller Loop, Water Tank and the Plate Handler.

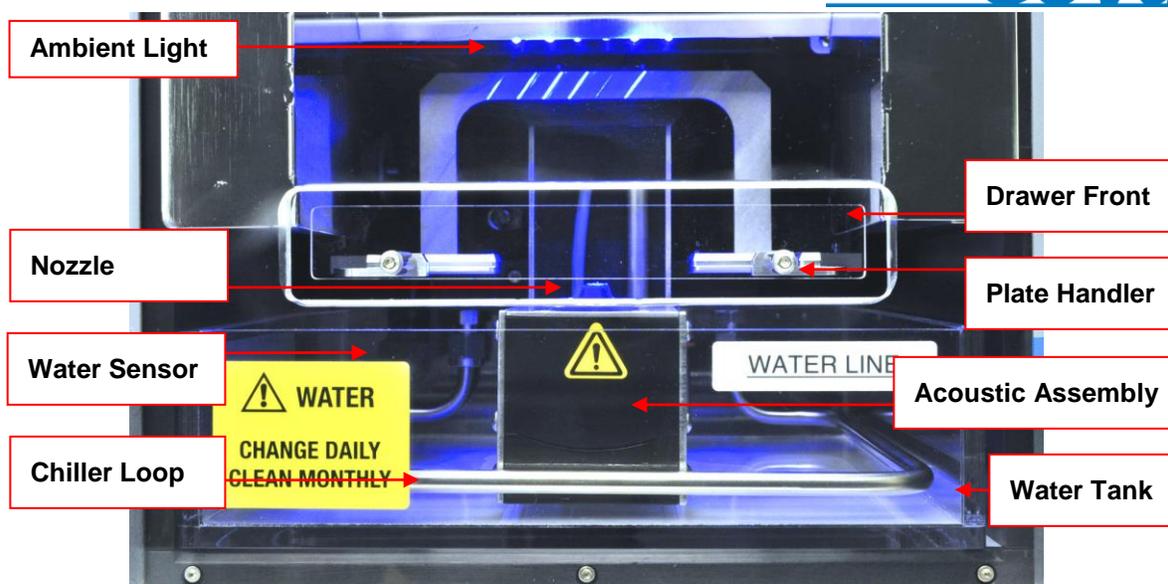


Figure 2 Front view and main components

3.3 Principle of Operation

The Covaris Process generates adaptive focused acoustic energy to cause precisely controlled cavitation and acoustic streaming along the focal line within the sample treatment microplate in a non-contact, isothermal process. Acoustic energy has been utilized for many years for a variety of diagnostic, therapeutic and research purposes. There are also specific clinical examples of the utilization of high intensity focused, therapeutic ultrasound (e.g. lithotripsy) and of low intensity, focused diagnostic ultrasound (e.g., fetal imaging).

The physics of ultrasonic energy is well understood; however, the biophysical, chemical and mechanical effects on biological systems are often only empirically understood. Well-known uses of sonic or acoustic energy in materials processing include "sonication", an uncontrolled process of mechanical disruption involving the direct immersion of an unfocused kHz range (i.e., 15,000 cycles per second) ultrasound source into a fluid suspension of the material being treated.

Typically, when ultrasound is applied to a bulk biological sample solution (as for the extraction of intracellular constituents from tissue), the treatment causes a complex, heterogeneous, mixture of sub-events that vary during the course of a treatment dose. In other words, the acoustic energy effects may change between various spatial and temporal states during the course of a treatment. For example, the energy may directly treat a sample; the energy may displace the target and shift the target out of the optimal energy zone; the energy may result in interference, i.e., a "bubble shield", that reflects the acoustic energy; or the large particles may move to low energy nodes thereby leaving the smaller particles with more dwell-time in the high energy nodes. In addition, the sample viscosity, temperature, and uniformity may vary during the ultrasonic process, resulting in gradients during the process. The current low frequency, unfocused sonicator-type processes are random and non-uniform. This limits the use of sonication in applications where treatment standardization is required.

In contrast, with the Covaris Process, focused acoustics are computer generated and delivered to a column of sample wells with the L-series system. The mechanical energy imparted on the sample results in a controlled series of compression and rarefaction events. The L-series instrument was designed to provide the researcher with methods and systems to control the delivery of mechanical energy into either a chemical or biological sample for multiple sample preparation and mixing applications.

Operating Mode

Frequency Sweeping Mode: This is the operating mode for the L8 instrument. The acoustic frequency sweeps through a range during processing. This mode delivers effective power and mixing to the sample.

3.4 Treatment System

3.4.1 Acoustic Assembly

The Acoustic Assembly houses the acoustic transducer, with an aperture and nozzle to direct a small fountain of water against the underside of the plate to be processed. A plate holder arm positions the plate over the Acoustic Assembly. The clear cover and plate holder arm incorporate safety interlocks that turn off the acoustic power when raised or open.

3.4.2 Acoustic Transducer

The acoustic transducer delivers a high intensity, focused, computer-controlled acoustic shock wave to each column of a microplate as the “treatment”. The intensity, duty cycle and burst pattern of each treatment is controlled by the SonoLAB software.

WARNING: Objects in the focal zone of the transducer will be exposed to high energy when the system is activated.

3.4.3 Temperature Sensors and Temperature Control

The water bath sensor, a 5 cm metal probe, monitors the temperature of the water bath and reports this on the Main Panel of the User Interface. The water bath temperature is controlled by attaching an external recirculating chiller/heater to the ports in the back of the system. The recirculator will pump a temperature-controlled fluid through a stainless steel loop immersed in the water bath. The user may set a maximum bath temperature in the User Interface to any value between 5° C and 40° C. If the bath temperature exceeds this limit, an indicator will be lit in the Main Panel and a fault notice will be posted to the user.

NOTE: Although the SonoLAB L-series software reports the bath temperature via the Temperature Sensor, it does not control the recirculating chiller. Typically, for most applications (e.g., gene expression, proteomics, compound dissolution, and drug metabolism studies) the temperature of the recirculating chiller is set to 17° C with the temperature of the water bath equilibrating at 20° C and the software temperature alarm set to a value slightly higher (e.g. 23° C).

NOTE: A recirculating chiller is required for efficient and controlled operation of the L-series instruments.

3.4.4 Water Bath and Degassing System

The water bath holds approximately 0.5 gallons (1.8 liters) of distilled or deionized water. The water bath must be degassed for proper operation of the instrument. The degassed, temperature controlled water provides an efficient energy couplant from the transducer to the sample to be processed. The water bath must be degassed for proper operation of the instrument.

The degassing system consists of a dip tube with six small holes, a positive displacement pump, and an outlet tube. Dissolved gasses and vapors are reduced in the water by pulling the water through the small holes in the dip tube and pumping the resulting coalesced bubbles out the outlet tube before they can re-dissolve. During normal operation, there should be an intermittent stream of bubbles coming from the hole at the end of the outlet tube. No bubbles indicate that some of the six small holes in the dip tube may be plugged, typically if the water bath becomes contaminated with particulate materials. If this occurs, the dip tube may be removed and the holes cleared with a fine wire (e.g., 26 Gauge needle).

CAUTION: The pump should not be run with the acoustic assembly raised or for a prolonged period with a fault condition present in the system. The degassing pump will turn off after 10 seconds if a low water condition is detected.

3.4.5 Fountain Pump

The fountain pump circulates water from the water bath into the Acoustic Assembly and out of the nozzle. The water being pumped into the Acoustic Assembly serves two purposes:

1. It keeps the transducer submersed in water, protecting it.
2. It exits out the nozzle on the top of the Acoustic Assembly creating a column of water that makes contact with the bottom of the microplate to couple acoustic energy into the plate.

The fountain pump will run whenever the degas pump is running.

CAUTION: Do not start a method with insufficient water level. This may cause the fountain pump to pull in air, possibly damaging the pump and reducing the effectiveness of the water column to transfer acoustic energy into the plate.

3.4.6 Computer

The computer uses the Microsoft™ Windows® Operating System with SonoLAB L-series software application.

NOTE: Power on the L8 instrument before starting SonoLAB L-series software. Communication via the USB cable must be established with the instrument for the software to operate.

NOTE: Any changes made to the application software will void the warranty. Please contact Covaris Technical Support for further information.

CAUTION: The “Tapping” option of the touchpad on the laptop, when enabled, may cause unexpected selection of buttons in the SonoLAB L-series software if the cursor is over a button and the touchpad is accidentally tapped. Disabling this option is recommended.

To disable the “Tapping” feature, perform the following:

1. From the Windows START button, select Settings > Control Panel.
2. Open Mouse properties.
3. De-select the “Tapping” option. See 0.
4. Click Apply.
5. Click OK to close the window.
6. Close the Control Panel.

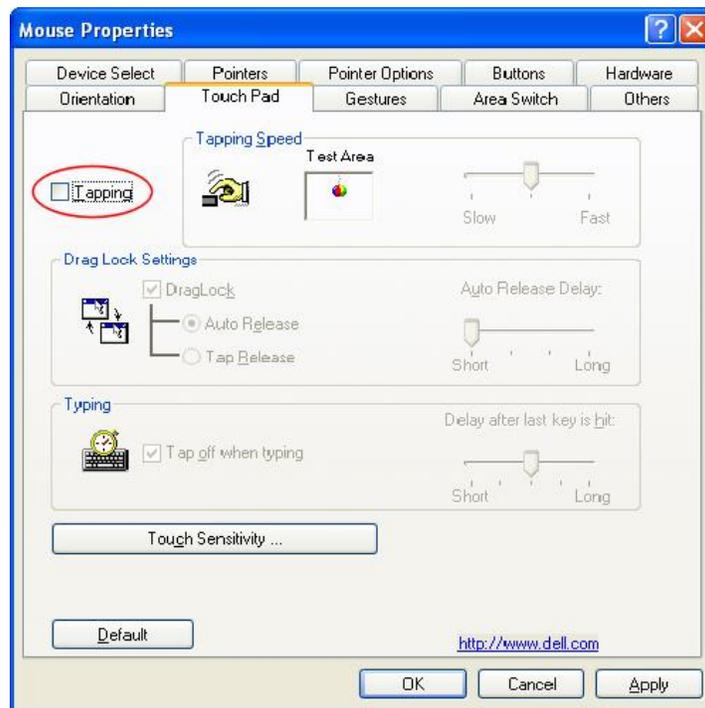


Figure 3 Disabling the Tapping feature

3.5 Safety System

The safety system protects the user from hazards associated with the acoustic treatment system. The safety system prevents generation of acoustic power if the Clear Cover is opened; the plate holder is out or water level is insufficient.

Clear Cover

The Clear Cover, when opened, will trip an interlock that stops all mechanical motion and turns off the acoustic treatment system. The opening of the Clear Cover should be limited to servicing the water bath.

CAUTION: Care should be taken when attempting to open the Clear Cover. Allow the handler to completely retract into the instrument and all motions to complete before attempting to open the Clear Cover or damage to the handler assembly may result.

CAUTION: Do attempt to open the Clear Cover after the EXTEND PLATE button has been selected as this may cause the Plate Handler to hold the Clear Cover open, tripping the safety interlock, and causing a situation where retracting the Plate Handler is necessary but impossible. If this occurs, perform the following steps:

1. Exit the SonoLAB software without moving to the Service position.
2. Power down the instrument.
3. Ensure there are no microplates on the handler.
4. Gently push the Plate Handler completely back into the instrument.
5. Close the Clear Cover.
6. Power up the instrument.
7. Launch the SonoLAB software and allow initialization of the instrument.

Plate Handler Drawer Front

Access to the inside of the unit is prevented by this drawer front when the plate handler is retracted. This protects the user from the hazards associated with the acoustic treatment system.

3.6 Application Software

The SonoLAB L-series software application controls all aspects of the L8 system. Full control of the L8 system is given through this software application. The following sections detail the software application's functionality.

4.0 SYSTEM OPERATION

4.1 Start-Up Procedures

4.1.1 Water Bath Configuration

Lift the Clear Cover, pull the water tank out part way and fill the water bath to the level indicated on the tank. The water level will rise when the Acoustic Assembly is lowered. Only distilled or deionized water should be used to fill the water bath.

CAUTION: Using anything other than distilled or deionized water in the water bath is a misapplication of the equipment and will void the warranty.

4.1.2 Water Bath

The system will determine if there is adequate water in the tank prior to starting a process. When the tank is properly filled, and the Acoustic Assembly is lowered, the water level will be approximately ½ inch (1.3cm) below the rim of the tank. Only distilled or deionized water should be used in the tank.

Special care needs to be taken when changing the water bath.

1. Verify the degassing pump has been turned “off”.
2. Remove any microplate that may be on the handler.
3. Select the SERVICE BATH button on the Main Panel.
4. Select OK and allow all motion to complete.
5. Wait a few minutes for water to drain from the acoustic assembly into the water tank.
6. Carefully pull out the acrylic water tank from the instrument base plate, empty, and wipe dry with lint-free cloth. The water fill line is marked on the front of the water bath:

WATER LINE

7. Fill the water tank to the Water Line on the front of the tank and place it back into its position, ensuring that it is oriented properly in the instrument.
8. If needed, empty and dry the drip tray that is under the water tank.

CAUTION: Do not overfill as the water may overflow during set-up operation. The water level will rise about ½ inch (1.3 cm) when the Acoustic Assembly is lowered.

4.1.3 Powering Up the System

Power on the Covaris L8 system with the switch located on the front of the instrument. The switch will glow red when the instrument is powered on. The front panel indicator lights will be lit when the instrument is powered on by itself. See 0.



Figure 4 Front panel indicator lights.

Power on the computer and open the SonoLAB L-series software by double clicking the SonoLAB L-series icon on the desktop. It may take up to 20 seconds for the USB link to be established.

The status of the front panel indicator lights are defined in Table 1.

Table 1 Indicator lights definition table.

Indicator State			System State
Blue	Yellow	Green	
Off	Off	Off	System off.
Steady On	Steady On	Steady On	System on, no software control.
Steady On	Steady On	Off	System Initialization in progress.
Steady On	Flashing	Off	User interaction requested.
Steady On	Off	Steady On	System is idle and ready.
Steady On	Off	Flashing	Method or motion is in process.

NOTE: If the computer is supplied by the user, follow the software installation procedure in Appendix B.

NOTE: Do not disconnect the USB cable or power off the L8 instrument while the SonoLAB L-series software is running. Loss of communication with the instrument may cause the software to become unresponsive, forcing the user to close the application via Windows Task Manager.

4.1.4 Interior Light

For light sensitive samples, it may be desirable to turn off the blue Interior Light. This may be accomplished by reaching behind the upper left of the instrument and toggling the Light switch to the off position.

4.1.5 Initialization

After communication has been established between the SonoLAB L-series™ software and the L8 instrument, an initialization dialog will be displayed as shown in 0.

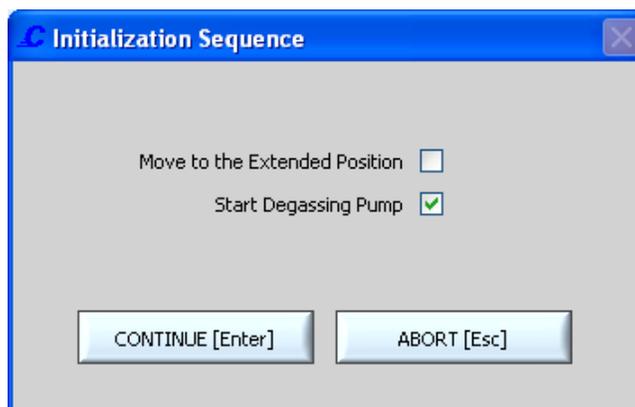


Figure 5 System start-up dialog with initialization options

By default, system initialization will:

1. Initialize and home the Acoustic Assembly and the Plate Handler.
2. Start the Degassing and Fountain pumps.
3. Move the Plate Handler to the Extended position, if selected.

The Acoustic Assembly and the Plate Handler will always home during system initialization and prior to displaying the Main Panel of the SonoLAB L-series software.

You may bypass any or all two available options by “unchecking” the desired option(s).

Select CONTINUE to proceed with system initialization and display the Main Panel.

Select ABORT to:

- Cancel system initialization and exit the software.
- Cancel system initialization in progress and exit the software

After system initialization is complete, the Main Panel of the software will be displayed. See 0.

4.2 Operating Procedure

4.2.1 Main Panel Screen

The Main Panel screen (see 0) allows you to:

- Select a previously created method (the selected method name appears at the top of the screen) and its associated parameters.
- Turn the degassing and fountain pumps on and off.
- Extend and retract the microplate handler.
- Service the water bath.
- Home the stage.
- Start a method.
- Go to the Method Editor to create or edit an existing method.
- Exit the software.

The Main Panel also contains Status indicators that allow you to monitor the activity and state of the system.

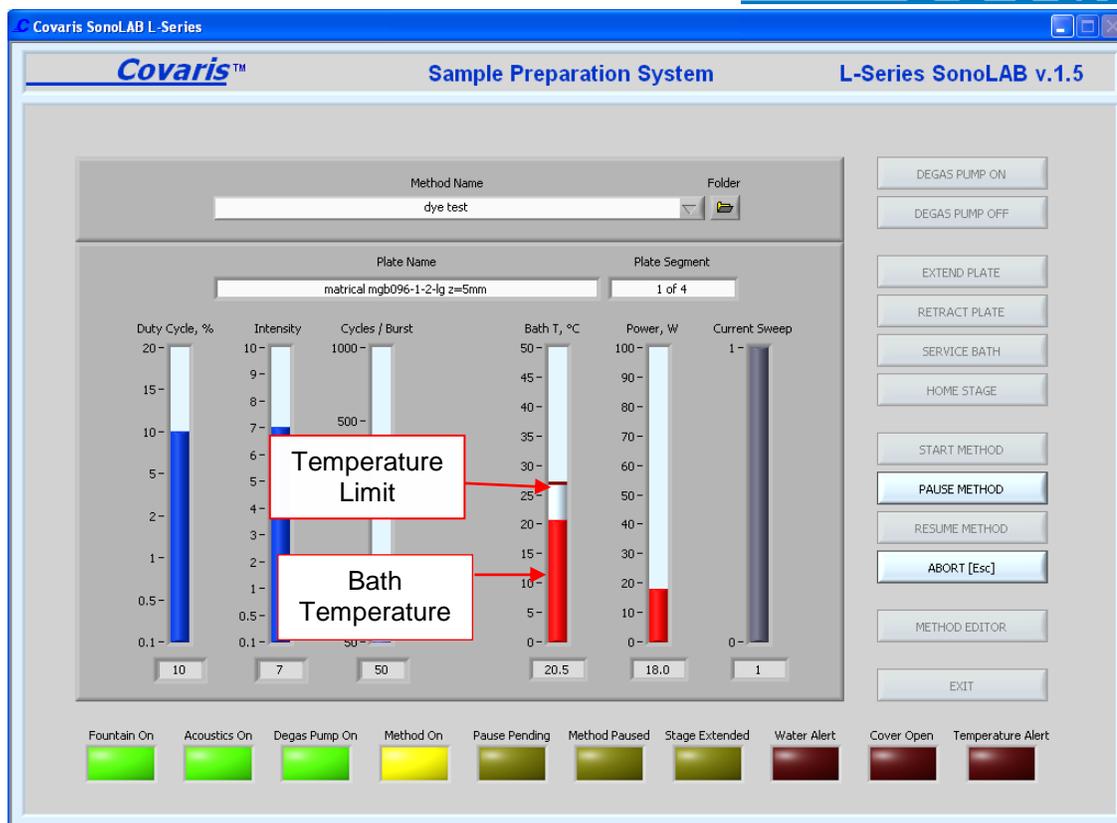


Figure 6 Main Panel of software application

The Status Indicators:

Method Name	Displays the selected method
Plate Name	Displays the Well Plate definition chosen for the selected Method.
Plate segment	Displays the segment of the plate that is currently being treated when a method is in process
Duty Cycle	The Duty Cycle, as a percentage, that is set in the selected Method.
Intensity	The Intensity level that is set in the selected Method.
Cycles/Burst	The Cycles per Burst that is set in the selected Method.
Bath T	Displays the temperature of the water bath and the upper limit set for the method. Bath temperature is displayed in degrees Celsius.
Power	Displays the power being applied to the transducer. Power reading is displayed in watts.
Current Sweep	Displays the current sweep number of a method in process.
Fountain On	Indicator is lit green when the fountain pump is on.
Acoustics On	Indicator is lit green when the transducer is on.
Degas Pump On	Indicator is lit green when the degassing pump is on. See Section 4.2.3.
Method On	Indicator is lit yellow when a method is in process.
Pause Pending	Indicator is lit yellow after the Pause button is pressed and the instrument is waiting for treatment of the current segment to complete.

Method Paused	Indicator is lit yellow when the current method is paused.
Stage Extended	Indicator is lit yellow when the Plate Handler is not fully retracted into the instrument. Use the RETRACT PLATE button to retract the Plate Handler.
Water Alert	Indicator is lit red when the water level is too low for treatment to occur. The transducer is disabled and degassing and fountain pumps turn off after a short delay when the system is in this condition. See Section 4.1.2.
Cover Open	Indicator is lit red when the clear cover is opened. All motion and the transducer will be disabled in this condition.
Temperature Alert	Indicator is lit red when the temperature of the water bath exceeds the Max Temperature setting in the Method. A warning is displayed when the water bath temperature exceeds the Max Temperature setting while a method is in process. The system is put into pause mode. You may override it and resume processing or you may stop the current method. See 0.

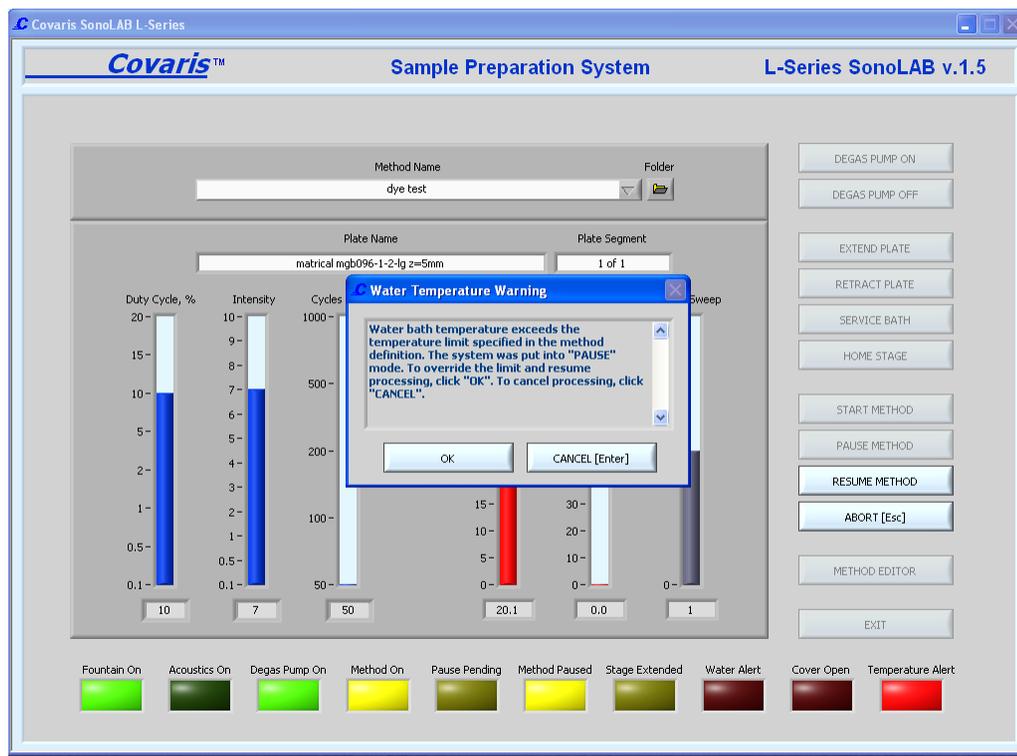


Figure 7 Water bath temperature warning

General Operation Buttons:

Method Pull down		Displays the list of Methods in the METHODS folder.
Folder		Allows the user to select the folder from which Methods are selected and saved. Navigate to the desired folder and open it to view its contents. Click the SELECT CUR DIR button to select the current folder being viewed. Only use the SELECT CUR DIR button to select the folder. See 0.

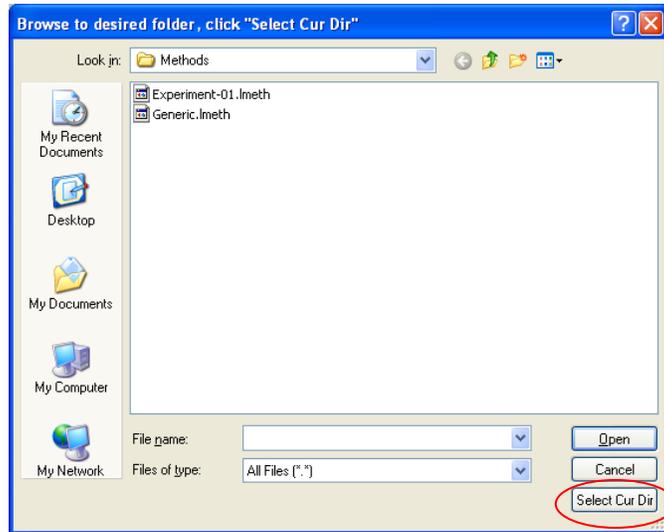


Figure 8 Method Folder selection with SELECT CUR DIR button

General Operation Buttons:

DEGAS ON	Is only active when a method is not in process and the degassing and fountain pumps are off and allows the user to turn them on.
DEGAS OFF	Is only active when a method is not in process and the degas and fountain pumps are on and allows the user to turn them off.
EXTEND PLATE	Is only active when a method is not in process and moves the Plate Handler to the extended position to allow access to it.
RETRACT PLATE	Is only active when a method is not in process and allows the user to retract the Plate Handler into the instrument.
SERVICE BATH	Moves the Acoustic Assembly and Chiller Loop into position to allow access to the Water Tank to service the water bath. The degassing and fountain pumps will shut off automatically and reset the degas timer.
HOME STAGE	Is only active when a method is not in process and allows the user to home the stage.
START METHOD	Initiates running a method after the plate handler is retracted.
PAUSE METHOD	Is only active after a method begins and allows a user to pause a method. The pause will occur at the end of the current sweep. Note: Do NOT pull on the Plate Handler. This will cause the registration of the handler to the nozzle to be lost and the method to abort.
RESUME METHOD	Is only active when a method has been paused and allows the user to resume that method.
ABORT	Is only active after a method begins and allows the user to stop the method at any time. The method cannot be restarted after this button has been selected.
METHOD EDITOR	Opens up the Method Editor where the user can modify or create a new method. See 0.
EXIT	Initiates shut down of SonoLAB L-series software application.

4.2.2 Method Editor Screen

The Method Editor allows you to define parameters of a treatment your samples will undergo. You may create a new method or edit existing methods.

A method consists of a treatment, which is a delivery of acoustic energy to the samples, given during each sweep cycle. The number of sweep cycles can be set from 1 to 1000. Duty Cycle, Intensity, and Cycles per Burst are acoustic Treatment Parameters.

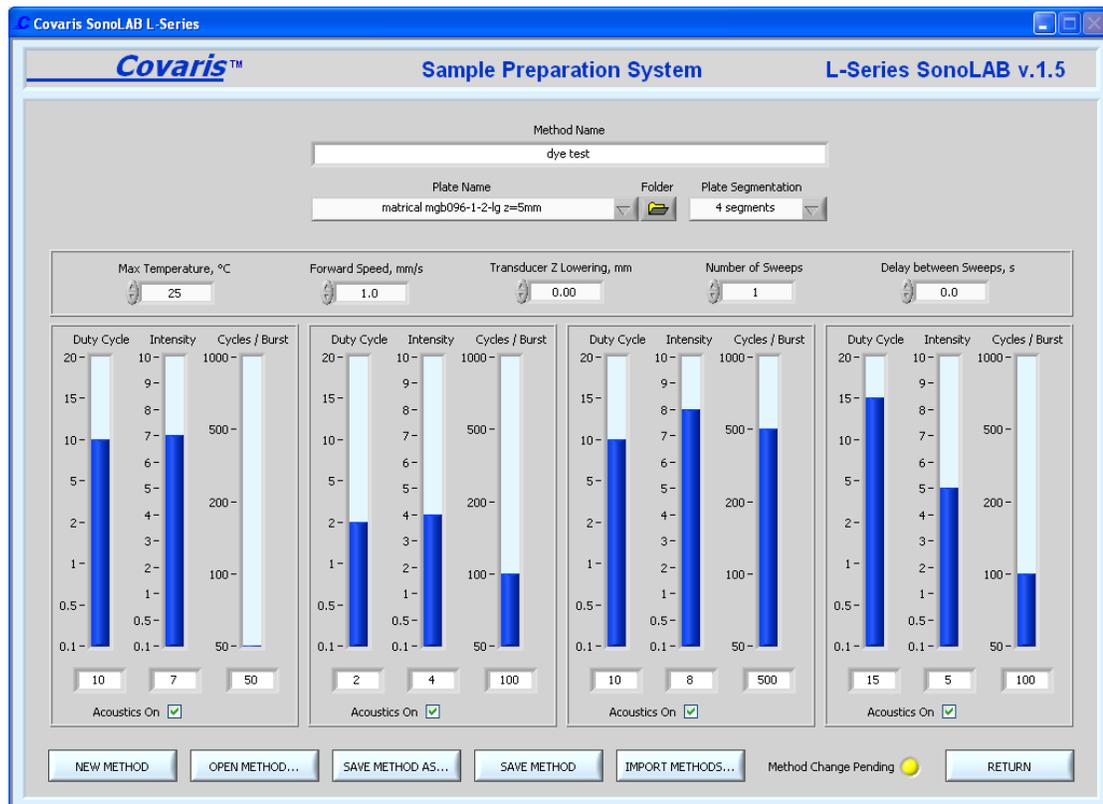


Figure 9 Method Editor

The Status Indicators:

Method Name	Displays the selected method
Plate Name	Displays the Well Plate definition chosen for the selected Method.
Plate segmentation	Allows the user to select how many segments the microtiter plate will be divided into, selection choices are from 1 to 4. The appropriate number of segments will be displayed and the user can set a different treatment method for each segment.
Method Change Pending	Indicator is lit yellow when changes made to the method have not been saved.

Plate Treatment Parameters:

Max Temperature	Allows the user to select a maximum temperature limit in degrees Celsius for the treatment. See Section 3.4.3
Forward Speed, mm/s	The speed in millimeters/second at which the Acoustic Assembly moves in the forward motion, from left to right. Treatment of the plate will be done during the forward motion and the speed should be set to minimize heating of the microtiter plate. Minimum is 0.2 mm/s, maximum is 15 mm/s. The speed in the reverse direction is fixed at 15 mm/sec.
Transducer Z Lowering, mm	The user can lower the transducer up to 1 millimeter from the position defined in the plate definition.
Number Of Sweeps	The desired number of sweep cycles to account for a full treatment. 1 to 1000 is permitted.
Delay Between Sweeps	The desired delay, in seconds, to impose between sweep cycles. Minimum is 0.

Segment Treatment Parameters:

Duty Cycle	Is defined as a percentage. For example, within a timeframe it is the percentage of the time that the transducer is creating acoustic waves; the “on” cycle. Typically, the higher the Duty Cycle the higher the number of acoustic waves per unit time is generated by the transducer. Minimum is 0.1% and Maximum is 20%.
Intensity	The amplitude of the pressure waves created by the acoustic transducer is proportional to the intensity. Essentially, the larger the number, the more energy each acoustic wave has. Minimum is 0.1 and maximum is 10.
Cycles Per Burst	The number of pressure waves generated by the transducer in a burst. For example, 100 Cycles/Burst at 10% duty cycle is a wavetrain of 100 “on” energy cycles followed by 900 “off” energy cycles. Minimum is 50 and Maximum is 1000.
Acoustics On	This is a check box to determine if the acoustics is on in a given segment. At least one segment of a method must be checked on.

General Operation Buttons:

Plate Pull down		Displays a list of Microtiter Plate definitions available to apply to the Method.
Folder		Allows the user to select the folder from which Well Plates are selected and saved. Navigate to the desired folder and open it to view its contents. Click the SELECT CUR DIR button to select the current folder being viewed. Only use the SELECT CUR DIR button to select the folder. See Figure 10.
New Method		Allows the user to create a method starting with default method parameters. The user will then be able to save method under a desired name.
Open Method		Allows the user to select a method from the list of previously created methods. The selected method will appear in the method name field.
Save Method		Allows the user to save a method set-up to the current method name as an *.LMETH file (in the Methods folder).
Save Method As		Allows the user to save the active or current method to an existing or new filename.
Return		Closes the Method Editor panel and returns to the Main Panel.

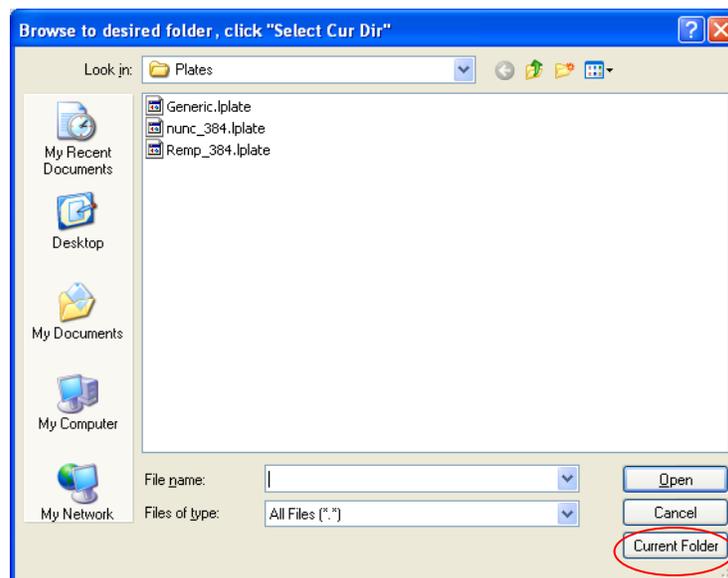


Figure 10 Plate Folder selection with select CURRENT FOLDER button

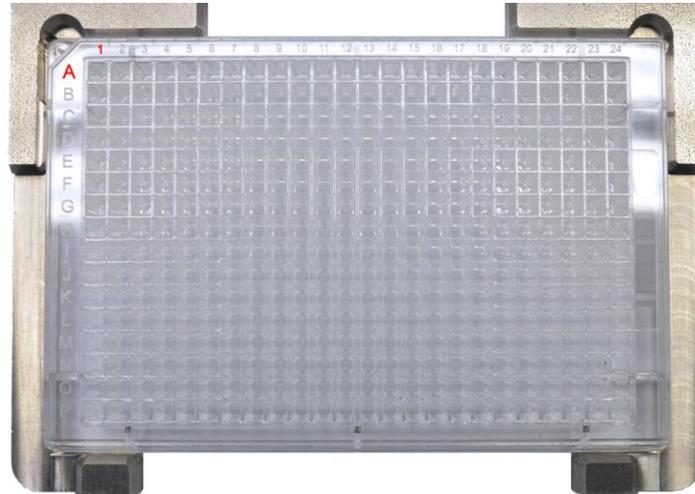
4.2.3 Degassing

The water bath must be degassed for proper operation of the instrument. Prior to running a method, degas the water bath for 10 minutes. The degassing system is engaged by pressing the DEGAS “ON” button in the Main Panel when the transducer is in the lowered position. The Degas Pump On indicator will be lit green to denote the pump is degassing the system.

NOTE: The degassing and fountain pumps will turn off after 10 seconds if the water tank is not filled with water to the Water Line.

4.2.4 Loading the microplate

Click the EXTEND PLATE button from the Main Panel. Load the microplate onto the handler so that well A1 is towards the upper left corner of the instrument.



NOTE: Ensure the microplate is seated completely in the handler so that it is coplanar with the acoustic assembly.

CAUTION: Do not attempt to open the Clear Cover after the EXTEND PLATE button has been selected as this may cause the Plate Handler to hold the Clear Cover open, tripping the safety interlock, and causing a situation where retracting the Plate Handler is necessary but impossible. If this occurs, perform the following steps:

1. Exit the SonoLAB software without moving to the Service position.
2. Power down the instrument.
3. Ensure there are no microplates on the handler.
4. Carefully push the Plate Handler completely back into the instrument.
5. Close the Clear Cover.
6. Power up the instrument.
7. Launch the SonoLAB software and allow initialization of the instrument.

4.2.5 Selecting the Method

Select the pull down arrow under Method Name and select the desired Method from the list.

4.2.6 Processing the Plate

Click the RETRACTPLATE button to retract the microplate and Plate Handler prior to starting a method. The Plate Handler must be retracted prior to starting a method or a warning message will be displayed. See 0. Click the START METHOD button on the Main Panel.



Figure 11 Retract Plate reminder dialog

CAUTION: Never run a method with an insufficient amount of water in the water tank; this will permanently damage the transducer.

After clicking the START METHOD button, the following dialog box will appear if the degassing pump has not run for 10 minutes or if the degassing pump has been turned off.

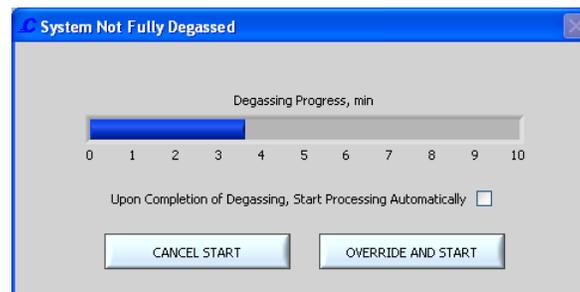


Figure 12 System Not Fully Degassed dialog and options

You may select the START PROCESSING AUTOMATICALLY checkbox and allow the method to start automatically after degassing is complete, CANCEL START and run the pump for additional time or OVERRIDE AND START to continue processing. The method will run until completion unless the ABORT button is pressed.

NOTE: Do not open the Clear Cover to stop the method or use the ABORT button by itself to cut the treatment short. To keep the treatment uniform across the microplate, use the PAUSE button to allow the treatment to complete its current sweep. Then use the ABORT button to stop the method.

An Error dialog will be displayed if there is an interlock fault and an attempt to start a method is performed or while a method is in progress. See Figure 13 and Figure 14. The interlock fault condition must be resolved or the Error dialog will reappear.

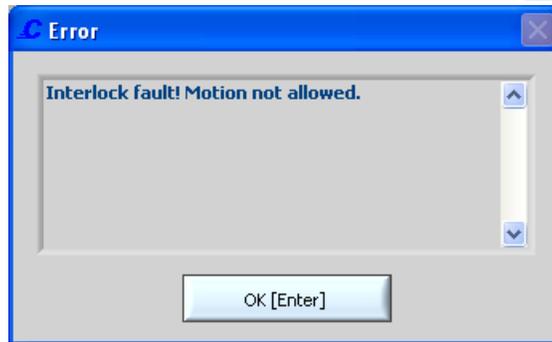


Figure 13 Interlock fault at start of method



Figure 14 Interlock fault during method in progress

4.2.7 Processing Complete

After the completion of a method, a dialog is displayed with the option to retrieve the microplate or to leave it inside the instrument. See 0. Select the OK button to retrieve the microplate. Select the CANCEL button to leave the microplate inside the instrument. You may use the EXTEND PLATE button to retrieve the microplate at a later time.

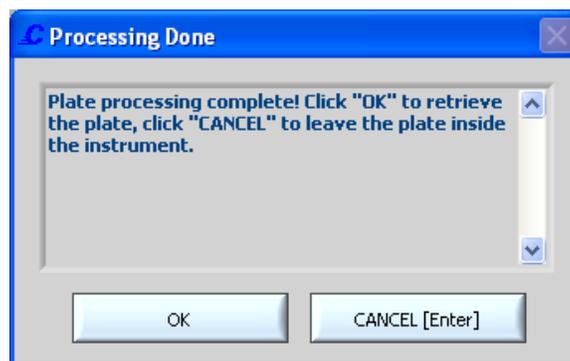


Figure 15 Plate processing complete dialog

4.3 Shutdown Procedure

If the instrument will be idle for an extended period of time, the degassing and fountain pump lines should be purged using the instructions in Sections 4.3.1 and **Error! Reference source not found.** If the system will be idle for a short period of time, proceed to Section 4.3.2 to shutdown the instrument.

4.3.1 To purge the degassing and fountain pumps:

1. Select the SERVICE BATH button from the Main Panel.
2. Ensure there are no microplates on the handler and select OK.
3. Allow the system to move to the Service Position.
4. Press the DEGAS ON button in the Main Panel.
5. Click OK to the warning dialog (see 0).

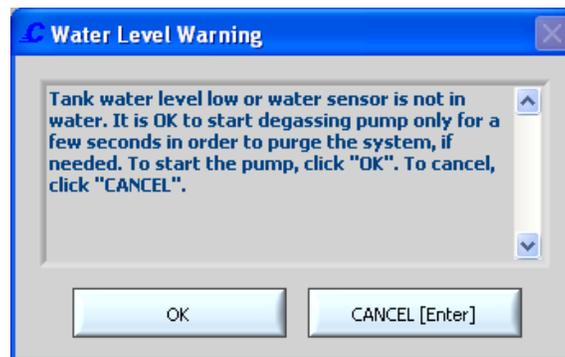


Figure 16 Water level warning dialog for purging the degassing pump

6. Run the pump for about 5 seconds.
7. Select the DEGAS OFF button.
8. Wait a few minutes for water to drain from the Acoustic Assembly into the water tank.
9. Empty the water bath and wipe the tank dry with a lint-free cloth.
10. Replace the water tank.

CAUTION: Leaving water in the water bath or degassing and fountain pump lines may allow biological (e.g., algae) growth in the tank and lines which will interfere with sample processing.

4.3.2 To shut down the instrument:

1. Close all screens in the SonoLAB L-series software.
2. Select the EXIT button from the Main Panel.
3. Ensure there are no microplates on the handler.
4. Select EXIT in the Exit Sequence dialog box/
5. Allow the Acoustic Assembly to move to the Service Position.
6. Power off the instrument after the SonoLAB L-series software has closed.
7. Power off the instrument using the switch located on the front of the instrument.

4.3.3 To shut down the laptop

1. Select TURN OFF COMPUTER from the START menu on the Windows screen.
2. Select the option TURN OFF.
3. Allow Windows to go through its shutdown sequence. The computer will power down.

5.0 SYSTEM SPECIFICATIONS

- Model:** L8
- Treatment System:** Bench-top; high intensity acoustic transducer with motion system for scanning microplates, microplate positioning system, temperature monitoring device, circulation pump, water bath with safety enclosure.
- Dimensions:** 15" W x 17" D x 14" H (38cm x 43cm x 36cm)
- Weight:** approximately 50 lbs (22.7 Kg)
- Power Requirements:** 100-240 VAC 300 VA, 50-60Hz
- Ambient Temp. Range:** 15 to 32°C
- Water Bath:** Distilled or deionized water only
- Water Temp. Alarm Limit:** Can be set at +5.0°C to +40.0°C
- Computer:** Notebook computer. Optionally supplied by Covaris.
- Operating System:** Microsoft Windows XP
- Application Software:** Covaris SonoLAB L-series
- Data Input:** Keyboard, mouse
- Chiller:** Chiller re-circulating system - not included. Connect with the 3/8 inch I.D. hoses and quick connect fittings supplied.
- Regulatory Labeling:** ETL Mark
- Safety:** Meets Low Voltage Directive 2006/95/EC.
Tested to EN/UL/CSA 61010-1:2004 "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements".



6.0 MAINTENANCE

6.1 Air intake

Periodically, unplug the unit and clean the air intakes (on each side of the instrument) to ensure that dust or dirt is not restricting the flow of air for component cooling. Otherwise, maintenance of the instrument should be performed under the direction of Covaris personnel.

6.2 Water Tank

6.2.1 Recommendations for Daily Maintenance

Only distilled or deionized water should be used to fill the water tank. There is a water line marked on the tank to determine the depth required.

The water tank should be emptied and wiped dry with a lint-free cloth at the end of every day to avoid algae growth. Do not use an algacide, alcohol or harsh cleaning chemicals.

CAUTION: Use of alcohol or harsh cleaning chemicals on the acrylic water tank may damage the acrylic. Use only clean water, or a bleach solution, and a lint-free cloth for water tank maintenance.

6.2.2 Recommendations for Monthly Maintenance

Periodically, the water tank, degassing and fountain pump lines may be rinsed with a **10%** solution of bleach (or equivalent, such as NaDCC). The resultant sodium hypochlorite solution is ~ 0.5% NaOCl. With this solution in the water tank, lower the transducer and run the degassing and fountain pumps for a few minutes. Repeat with fresh water in the water tank.

6.3 Transducer

When not in use, the transducer and acoustic assembly should be raised out of the water bath to the Service Position. Keeping the transducer dry between applications will help prevent metal corrosion and is strongly encouraged to ensure optimal performance.

Do not drop objects into the Acoustic Assembly onto the transducer, as these will interfere with the acoustic performance and may damage the transducer face.

Permanent damage could also result if the transducer is overheated, i.e. running the transducer with an insufficient amount of water in the water tank.

WARNING: Do not attempt to remove the transducer as irreparable damage may occur.

6.4 Safety System

Test the system periodically. Make sure that the acoustic power does not go on when the Clear Cover is opened or if the water level in the tank is too low.

WARNING: Any failure of the Safety System must be reported immediately to Covaris. DO NOT attempt operation if the Safety System is malfunctioning.

6.5 Pump, Hoses, and Cables

The degassing and fountain pumps should not be run without a water bath. This will cause the pump head to wear out.

Check that there are bubbles coming from the end of the degassing outlet tube. If there are no or few bubbles present, remove the dip tube, by pulling downward, and examine it under a microscope to clear any clogged holes (e.g., small pin or purge with compressed gas). Reinstall by pushing the dip tube up into the fitting. Be sure the tube is fully seated in the fitting.

For long-term storage, water should be removed from the degassing pump lines. Follow shutdown procedure in Section 0.

6.6 Relocation of the System

Should it be necessary to move the instrument, for whatever reason, the system needs to be properly prepared. Prior to this movement, make sure all steps on the following checklist are completed.

- Follow the shutdown procedure in Section 0.
- Save all files and close the SonoLAB L-series software by closing all screens and selecting EXIT on the Main Panel. Select EXIT in the dialog box.
- Select TURN OFF COMPUTER from the Windows START menu and make sure the option to TURN OFF is selected. The computer will power down. Turn off the monitor.
- Power down the instrument by moving the power switch to the OFF position. The power switch is located on the front of the instrument.
- Disconnect the cable from the computer to the instrument.
- Remove the water tank.
- Cover the system and computer with a light cloth or plastic to prevent dust contamination.

6.7 Cleaning the System

Clean the external surfaces of the equipment as necessary with a damp lint-free cloth. Rinse the water tank with clean water and wipe dry with a lint-free cloth.

CAUTION: Use of alcohol or harsh cleaning chemicals on the acrylic water tank may damage the acrylic. Use only clean water, or a bleach solution, and a lint-free cloth for water tank maintenance.

7.0 TROUBLESHOOTING

System Trouble Analysis Chart

SYMPTOM	PROBABLE CAUSE	POSSIBLE SOLUTION
Degassing system not generating bubbles. Samples take longer to process. Method is noticeably noisier.	Degassing dip tube may be clogged.	<ol style="list-style-type: none"> 1. Remove dip tube and back flush under running water. 2. Examine the six holes (e.g., using a stereo microscope) to see if clogged and use a pin to unclog them and/or purge with compressed gas.
	Pump not on or not working.	If pump does not run, contact Covaris tech support.
Safety interrupt panel is displayed.	Clear cover is not closed completely.	<ol style="list-style-type: none"> 1. Close the Clear Cover. 2. Verify presence of magnet in lower left of lid.
	Water level is too low.	Fill water tank to the fill line.
Instrument unable to communicate with hardware.	Computer was turned on without instrument on or connected.	Turn on instrument and select retry button.
	USB cable is loose or damaged.	Check the USB cable.
Software Application is unresponsive.	The USB becomes disconnected while the Application is running.	<ol style="list-style-type: none"> 1. Reboot the computer. 2. Terminate the Application; may need to use Windows Task Manager to terminate.
	The L8 is turned off while the Application is running.	<ol style="list-style-type: none"> 3. Turn off L8 instrument when the SonoLAB software is closed or See appendix B for standby and hibernate mode settings.
	The L8 is turned on when the laptop goes into standby or hibernate modes.	
Water Alert comes on when water tank is correctly filled.	Acoustic Assembly not fully lowered.	Verify Acoustic Assembly is down.
Error message that system not shut down correctly.	TURN OFF command in Windows XP not used before rebooting.	<ol style="list-style-type: none"> 1. Follow instructions on screen. 2. Use TURN OFF command.

APPENDIX A

Plate Definitions, Mixing and Sample Preparation

The system may be used with any micro-titer plate for which a plate definition has been created. Please contact Covaris for assistance with correct plate definitions.

The system may be configured for additional microplates, temperatures, and for use in robotic systems. Please contact Covaris for any additional questions or comments regarding system configuration, mixing and sample processing.

APPENDIX B

Procedure to install USB drivers and Covaris SonoLAB L-series software on a Windows XP computer

NOTE: Follow the instructions in sequence.

1. Begin with the USB cable disconnected and the instrument powered off.
2. Load the INSTALL CD onto the Laptop's CD-Drive. The SonoLAB installer will launch in about 30 seconds. If the SonoLAB installer does not launch automatically, navigate to the CD drive and double-click on the Setup application file to start the installation process.
3. Simply click Next to proceed through the installation process. When queried, accept any software license agreement and click Next to continue with the installation.
4. When installation is complete, click Finish and leave the CD in the CD-Drive.
5. Power on the instrument and plug the USB cable into a USB port on the computer.
6. The Windows hardware INSTALL Wizard will start. Choose "No not this time" when queried about connecting to Windows Update. If Hardware Installation warnings appear, click "Continue Anyway" to proceed with the installation. The wizard will run four times.
7. When the driver installation is complete, the system should be ready to use.
8. For convenient usage, find the executable file under C:\Covaris\L-Series v1.x\Program and create a shortcut on the desktop (Do not drag the executable file to the desktop, just "create shortcut".)
9. Double click on the shortcut to start SonoLAB L-series software.

NOTE: Laptop standby and hibernate modes should be avoided while operating the SonoLAB L-series software. To ensure this, from the start menu, open Control Panel, then Power Options. Select a Power Scheme that sets System standby and System hibernate times to Never. If standby and hibernate modes remain enabled, then the SonoLAB L-series software should be closed and the system powered down whenever the system is not in use.

NOTE: SonoLab L-series software was not tested with other application software and therefore we recommend that no additional application software is loaded. Wireless Network Connections on the Laptop should be disabled to avoid loading of any unwanted software. Go to Network Connections in the Control Panel. Highlight the Wireless Network Connection and select "disable this network device".

APPENDIX C

References

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety
- Botwell, D. and Sambrook, J. (2003) DNA Microarrays, A Molecular Cloning Manual, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, NY.