

EpiShear™ Multi-Sample Sonicator / Chiller

EpiShear™ Multi-Sample Sonicator

(version D1)

Sonicator / Chiller: Catalog Nos. 53062 (110V) & 53063 (230V)

Sonicator only: Catalog Nos. 53065 (110V) & 53066 (230V)

Active Motif North America

1914 Palomar Oaks Way, Suite 150
Carlsbad, California 92008, USA
Toll free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351

Active Motif Europe

Avenue Reine Astrid, 92
B-1330 La Hulpe, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733

Copyright 2012 Active Motif, Inc.

Information in this manual is subject to change without notice and does not constitute a commitment on the part of Active Motif, Inc. It is supplied on an “as is” basis without any warranty of any kind, either explicit or implied. Information may be changed or updated in this manual at any time.

This documentation may not be copied, transferred, reproduced, disclosed, or duplicated, in whole or in part, without the prior written consent of Active Motif, Inc. This documentation is proprietary information and protected by the copyright laws of the United States and international treaties.

The manufacturer of this documentation is Active Motif, Inc.

© 2012 Active Motif, Inc., 1914 Palomar Oaks Way, Suite 150; Carlsbad, CA 92008. All rights reserved.

All trademarks, trade names, service marks or logos referenced herein belong to their respective companies.

TABLE OF CONTENTS	Page
Overview	1
Safety Warnings	2
Technical Specifications	3
Principles of Operation	4
Included Components	6
Components Lists	7
Preparation for Use	8
Attach the Converter to the Cup Horn / Water Bath	9
Insert the Cup Horn / Water Bath into the Sound Enclosure	10
Functions of Keys, Controls, Indicators and Connectors	12
Operating Instructions	14
Sonication Shearing of Chromatin for CHIP	18
Appendix	
Section A. Troubleshooting	20
Section B. Overload Condition	20
Section C. Maintenance	20
Section D. Limited Warranty	24
Section E. Service / Return of Equipment	25
Section F. Safety Certification Form	25
Section G. Related Products	26
Technical Services	28

Overview

Active Motif's EpiShear™ Multi-Sample Sonicator is a high-intensity cup horn sonicator that can shear up to eight vials (20 µl - 1.2 ml of sample / vial) of chromatin or DNA simultaneously for use in chromatin IP (ChIP), DNA methylation studies and Next-Gen sequencing. It can also be used for standard cell disruption, RNA shearing and other homogenization applications. The EpiShear Multi-Sample Sonicator is manufactured in the United States, and is backed by a two-year warranty.

The sonicator is controlled by a microprocessor unit that offers both programmable and manual operation. The 750-watt generator has a keypad and digital display that make it easy to program the amplitude and to set the total sonication time, as well as the duration of the On and Off pulse cycles. It also makes it easy to monitor and change sonication parameters. The display also shows the total elapsed time, and provides real-time energy monitoring of both wattage and joules.

The unit utilizes a cup horn sonicator that is mounted inside a water bath, which is housed inside a compact sound enclosure that reduces the noise produced during sonication. The water bath and sound enclosure contain quick-connect hoses that make it fast and easy to attach the water bath to a thermoelectric chiller, which keeps the samples at 3-4°C during sonication. To ensure reproducibility, the samples rotate continuously in the water bath, so all samples are subjected to the same amount of sonic energy. The sound enclosure has LED lights inside and a small window that enables the samples to be monitored during sonication.

The EpiShear Multi-Sample Sonicator can be bought with or without our EpiShear Thermoelectric Chiller. As the EpiShear Multi-Sample Sonicator generates a substantial amount of heat during sonication, use of a chiller is imperative to obtain best results.

The EpiShear Multi-Sample Sonicator is supplied with an ultrasonic electric generator, a sound enclosure, a high-intensity cup horn piezoelectric converter, an acrylic water bath, an 8-sample microtube holder, an electronic sample rotator/illuminator, power cables, quick-connect water tubing, a wrench set and 200 Polystyrene Sonication Tubes. (Because polystyrene transfers sonic wave energy much more efficiently than polypropylene, Active Motif highly recommends that its Polystyrene Sonication Tubes be used with the Multi-Sample Sonicator. Use of other types of tubes have been shown to produce sub-optimal results.)

product	format	catalog no.
EpiShear™ Multi-Sample Sonicator / Chiller	110V 230V	53062 53063
EpiShear™ Multi-Sample Sonicator	110V 230V	53065 53066
Sonication Tubes / Caps, 1.5 ml polystyrene	200 tubes 1000 tubes	53071 53072

As this manual contains many images, we recommend that you download its PDF version from our website; the pictures will then be in color, and you will be able to zoom in for better detail.

Safety Warnings

PLEASE READ THE ENTIRE MANUAL BEFORE USE OF THIS DEVICE!

The EpiShear has been designed, built and tested to ensure maximum operator safety. However, no design can completely protect against improper use that may lead to bodily injury, death and/or property damage. For total safety and equipment protection, read the entire instruction manual carefully before attempting to operate this equipment.

Be certain to observe the following **WARNINGS**:

- High voltage is present in the generator (power supply), converter and high frequency cables. There are no user-serviceable parts inside any of these devices. **DO NOT** attempt to remove the generator cover or converter case.
- **DO NOT** touch any open cable connections on the unit while the power is turned ON.
- **DO NOT** operate the generator with the converter disconnected from the high voltage cable. High voltage is present in the cable and may pose a shock hazard.
- **DO NOT** disconnect the converter high voltage cable while the unit is running.
- The generator must be properly grounded with a 3-prong plug. **TEST** the electrical outlet you will be using for proper grounding before plugging in the unit.
- Install the unit in an area free from dust, dirt, explosive or corrosive fumes and protected from extremes in temperature and humidity. **DO NOT** place the generator in a Fume Hood.
- **NEVER** immerse the converter in liquids of any kind, or allow condensed moisture or liquid to drip into the converter.
- **NEVER** touch a vibrating cup horn. It can cause severe burns and tissue damage.
- **NEVER** allow a cup horn to vibrate in air (not immersed in liquid) for more than 10 seconds.
- Avoid touching the bottom or sides of glass or plastic containers with an activated cup horn. It could crack or shatter the glass or melt the plastic.
- Turn the power switch to OFF, unplug the generator and disconnect the power cord from the back of the generator before attempting to replace the fuses.
- Inspect the high frequency cables for cracks in the protective outer jacket.
- **DO NOT** operate the unit with a damaged cable. Doing so may cause serious injury.
- In case of an AC power loss, wait a minimum of 3 minutes before reapplying the power.
- **DO NOT** turn off the AC power while operating the EpiShear. Use the touch screen to stop sonication prior to turning off the AC power.

Technical Specifications

Generator	110V (Catalog No. 53062)	230V (Catalog No. 53063)
Input Voltage	100 VAC – 120 VAC @ 50/60 Hz	220 VAC – 240 VAC @ 50/60 Hz
Rated Current	10 Amps max.	5 Amps max.
Fuse Rating	15 Amps (Slow-blow)*	8 Amps (Slow-blow)*
Sample Size	One to eight 1.5 ml vials (20 µl - 1.2 ml sample / vial)	
Programmable Timer	1 second to 10 hours	
Adjustable Pulse	1 second to 59 seconds	
Adjustable Amplitude	20% - 100%	
Weight	14.0 lbs. (6.4 Kg)	
Dimensions	14.5" D x 9" H x 8" W (368 mm x 229 mm x 203 mm)	
Power Rating	750 watts	
Output Voltage	1000 V rms (max.)	
Output Frequency	20 kHz	

Cup Horn / Sound Enclosure	
Weight	29.5 lbs. (13.4 Kg)
Dimensions	13" D x 19" H x 11.5" W (330 mm x 483 mm x 292 mm)

Thermoelectric Chiller (see the Thermoelectric Chiller manual for more information)	
Weight	8.1 lbs. (3.7 Kg)
Dimensions	7.5" D x 7" H x 5" W (191 mm x 178 mm x 127 mm)

Environmental	
Pollution Degree	2
Installation Category	II
Operating Limits	Temperature: 41° - 104°F (5° - 40°C) Relative Humidity: 10% - 95% (Non Condensing) Altitude: 6,651 feet (2,000 m)
Shipping/Storage	Temperature: 35° - 120°F (2° - 49°C) Relative Humidity: 10% - 95% (Non Condensing) Ambient Pressure Extremes: 40,000 feet (12,192 m)
Restriction of Hazardous Substances (RoHS)	RoHS Compliant Directive 2002/95/EC
Relative Humidity	Maximum relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C.
Other	For indoor use only.

*Only use IEC-approved Slow-blow fuses, Cooper Bussmann.

The Power Cord supplied with the EpiShear must be used. If the 230V plug is not configured to match the wall receptacle, a properly grounded, universal AC socket adapter must be added.

Important: Universal adapters do not convert voltage or frequency. Active Motif is not responsible for damage caused by the use of an improper power cord or adapter. Transformers are not recommended.



WEEE Statement.

This product contains electrical or electronic materials. The presence of these materials may, if not disposed of properly, have potentially adverse effects on the environment and human health. Presence of this label on the product means it should not be disposed of as unsorted waste and must be collected separately. As a consumer, you are responsible for ensuring that this product is disposed of properly. To find out how to properly dispose of this product contact Active Motif's Technical Services.

Principles of Operation

In sonication, electrical energy is converted into physical vibrations (sound energy in the form of ultrasonic waves), which are used to process the sample. First, an electronic generator is used to transform conventional AC line power (50/60 Hz) into high-frequency electricity (20,000 Hz). The 20 kHz electricity then drives a piezoelectric converter/transducer. The electrical energy is converted by the transducer to mechanical vibration due to the characteristics of the converter's internal piezoelectric crystals.

The vibration is amplified and transmitted down the length of a probe or cup horn, which longitudinally expands and contracts at the tip. The distance the tip travels is dependent on the amplitude selected by the user through the unit's keypad. As you increase the amplitude setting, the sonication intensity will increase within your sample.

In liquid, the rapid vibration of the tip causes cavitation, which is the formation and violent collapse of microscopic bubbles. The collapse of thousands of cavitation bubbles releases tremendous energy in the cavitation field. The erosion and shock effect of the collapse of the cavitation bubbles is the primary mechanism of fluid processing.

Relationship of Amplitude and Wattage

Sonication power is measured in watts. Amplitude is a measurement of the excursion of the tip of the probe (the distance the probe moves away from and back to its point of equilibrium).

The ultrasonic processor was designed to deliver constant amplitude to your liquid sample, regardless of the changes in load. As a liquid is processed, the load on the probe/cup horn will vary due to changes in the liquid sample (*i.e.* viscosity, concentration, temperature, *etc.*). For example, the unit experiences a higher load when processing viscous samples as compared to aqueous samples.

During operation, the EpiShear generator displays the wattage, which is the energy required to drive the radiating face of the probe/cup horn at the specific amplitude setting against the load

being experienced at that instant. As the resistance to the movement of the probe/cup horn increases or decreases (changing the load), more or less power will be delivered by the power supply to ensure that the excursion at the probe/cup horn tip remains constant. Therefore, while the displayed wattage readings will vary as the load changes, the amplitude will remain the same.

Because of this, use of a higher-wattage generator does not automatically mean that more power will be transmitted to the liquid. And, programming the amplitude at 100% does not mean that the generator will deliver its maximum wattage. The amount of power delivered at any given moment will be only what is required to maintain the set amplitude.

To a certain extent, the speed/cruise control on an automobile can be compared to an ultrasonic processor. The speed/cruise control is designed to ensure that your vehicle maintains a constant rate of travel, or speed. As the terrain changes, so do the vehicle's power requirements to maintain the constant speed. If you have set your cruise control and begin to go up a hill, the engine must produce more power (RPMs, or Rotations Per Minute) to maintain the constant speed. The cruise control senses these requirements and automatically adjusts the amount of power delivered by the engine in order to compensate for the ever-changing conditions. Thus, in this example, wattage can be thought of as the engine RPMs and the amplitude as the constant speed that is maintained.

The resistance to the movement of the probe determines how much power will be delivered to maintain amplitude. For example, a 1/2" probe at 100% amplitude will require approximately 5 watts to operate in air. The amplitude of this probe is approximately 120 μm . After inserting the probe in water, the wattage reading will increase to approximately 90 watts. The wattage required to operate the probe will increase as the load increases, but the amplitude remains the same.

The AMPLITUDE control enables the ultrasonic vibrations at the probe tip to be set to any desired level. Although the degree of cavitation/ultrasonic energy required to process the sample can readily be determined by visual observation, the amount of power required cannot be pre-determined. A sensing network continuously monitors the output requirements and automatically adjusts the power to maintain the amplitude at the pre-selected level. The greater the resistance to the movement of the probe due to higher viscosity, deeper immersion of the probe into the sample, larger probe diameter or higher pressure, the greater the amount of power that will be delivered to the probe.

Setting the AMPLITUDE control to its maximum will not cause the maximum power rating of the unit to be delivered to the sample. The maximum power (750 watts) that the EpiShear Multi-Sample Sonicator is capable of delivering will only be delivered when the resistance to the movement of the probe is high enough to draw maximum wattage.

It is the intensity of cavitation that measures the effectiveness of the sonication, not the total power applied to the system. Intensity is directly related to the amplitude of the radiating face of the probe tip. It is amplitude that must be provided, maintained and monitored. The unit delivers controlled amplitude under varying load conditions in order to provide reproducible results.

Included Components

The EpiShear Multi-Sample Sonicator is supplied with an electric generator and a sound enclosure that houses a high-intensity cup horn / piezoelectric converter that mounts inside an acrylic water bath which holds an 8-sample microtube holder and an electronic sample rotator/LED illuminator (Figure 1). The unit can be supplied with or without the EpiShear Thermoelectric Chiller (right).

To simplify set up and reduce clutter, the interior of the sound enclosure is pre-wired with a high voltage cable to connect power from the generator to the converter and sample rotator, and pre-plumbed with quick-connect water tubing that is used to attach the water bath to the chiller. Power cables are supplied to connect to the generator to the rear of the enclosure, as is a wrench set for tightening/connecting the converter to the cup horn. A bag of 200 Polystyrene Sonication Tubes are also included.

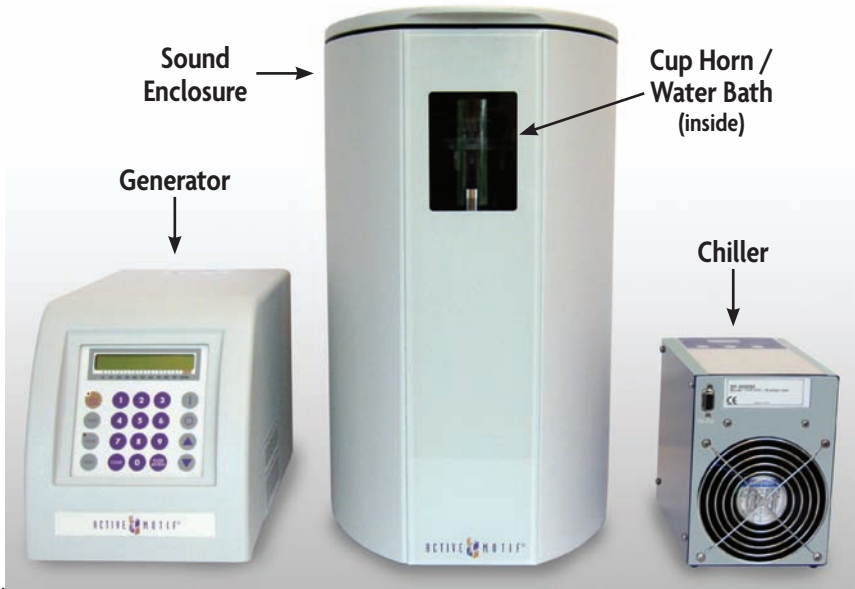


Figure 1: The EpiShear™ Multi-Sample Sonicator / Chiller.

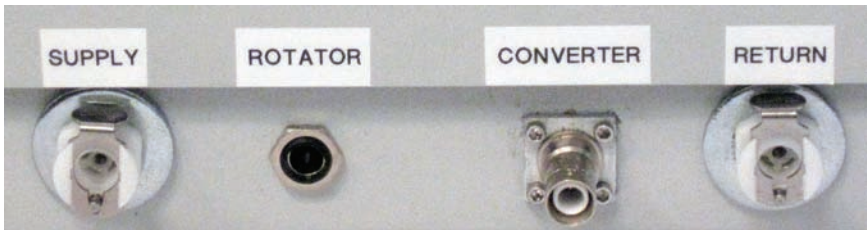


Figure 2: Placing the electrical connections and hose fittings on the rear of the sound enclosure makes set up simple.

Components Lists

Sonicator Components

- Power supply
- Converter cable
- Power cable
- Wrenches

Sound Enclosure

- Enclosure
- Rotator motor
- Rotator housing
- Rotator motor cable
- Tube rack with cover and post
- Internal tubing with fittings
- Internal cables

Chiller Components (optional)

- Chiller unit
- Power supply
- Power cable
- Set of inlet, outlet and drain tubes with quick-connect fittings attached

Cup Horn Components

- Acrylic water bath with fittings
- Titanium horn
- (2) O-rings
- Threaded bushing
- Converter



Preparation for Use

Inspection

Prior to assembly and installation of the EpiShear Multi-Sample Sonicator, perform a visual inspection to detect any evidence of damage that may have occurred during shipment. Before disposing of any packaging material, check it carefully for small items.

The EpiShear Multi-Sample Sonicator was carefully packed and thoroughly inspected before leaving the factory. The carrier, upon acceptance of the shipment, assumed responsibility for its safe delivery. Claims for loss or damage sustained in transit must be submitted to the carrier.

If damage has occurred, contact your carrier within 48 hours of the delivery date. **DO NOT OPERATE DAMAGED EQUIPMENT.** Retain all packing materials for future shipment.

Electrical Requirements

The EpiShear Multi-Sample Sonicator requires a fused, single phase 3-terminal grounding type electrical outlet capable of supplying 50/60 Hz at 100-120 volts, or 220-240 volts, depending on the voltage option selected. For power requirements, check the label on the back of the unit.



**Caution, Risk of electric shock.
Hazardous voltage.**



Caution, Risk of danger.

Warning: For your personal safety, do not, under any circumstances, defeat the grounding feature of the power cord by removing the grounding prong.

Installation

The EpiShear Sonicator should be installed in an area that is free from excessive dust, dirt, explosive and corrosive fumes, and extremes of temperature and humidity. If processing flammable liquids use an approved fume hood and **DO NOT** place the power supply in the fume hood.

Be sure to leave adequate space behind the unit so that all connections can be disconnected.

Attach the Converter to the Cup Horn / Water Bath

The Multi-Sample Sonicator is shipped with the cup horn already inserted into the water bath. You will first need to securely attach the converter to the cup horn / water bath assembly and then place it all inside the sound enclosure.

1. While the supplied water bath / cup horn should be water tight, it is only prudent to test this before proceeding. Fill the water bath / cup horn with water and check the bottom of the water bath for any leakage. After confirming that the assembly does not leak, remove the water and proceed with the next step.

On the off chance that the unit does leak, see the Assembly / Disassembly of the Cup Horn / Water Bath instructions in Appendix – Section C. These explain how to remove the cup horn from the water bath and then reassemble, which will need to be done periodically in any event to properly clean the unit. Ensure that the O-rings are compressed against the flange. If you have silicone grease you may wish to lightly coat each O-ring, as this will help ensure a good seal.

2. Using the provided wrenches, tightly attach the converter onto the cup horn / water bath. Do not hand tighten this connection; it is subject to considerable vibration, which will cause it to loosen. Properly tighten with the wrench set that was supplied with your unit. The image below shows the cup horn without the water bath to better illustrate this step.



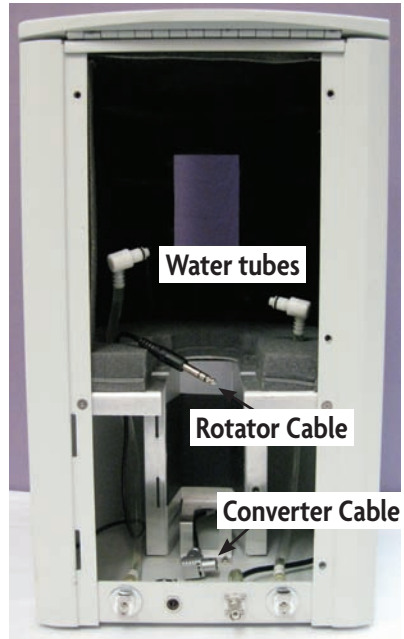
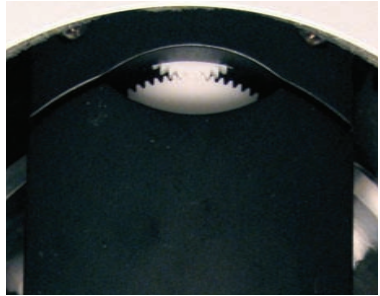
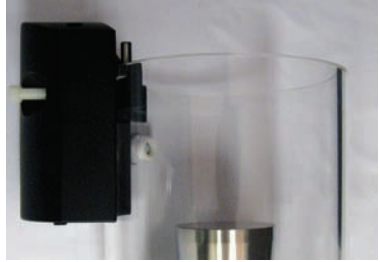
IMPORTANT: Be certain the cup horn and converter are sufficiently tightened. If this connection is loose, or works itself loose during use, the generator may display an OVERLOAD error.

3. Proceed with the next section to place the cup horn / water bath into the sound enclosure and connect the power cables and water tubing.

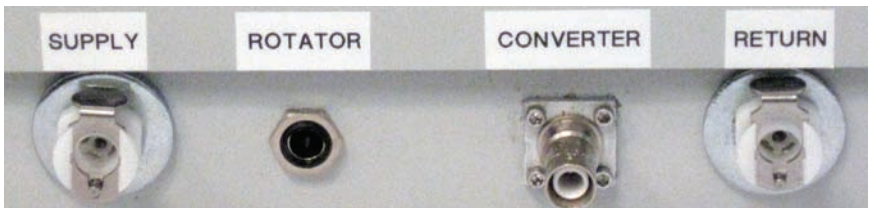
Insert the Cup Horn / Water Bath into the Sound Enclosure

After attaching the converter the cup horn/ water bath, it all must be placed inside the sound enclosure, and the power cables and water tubing must be attached.

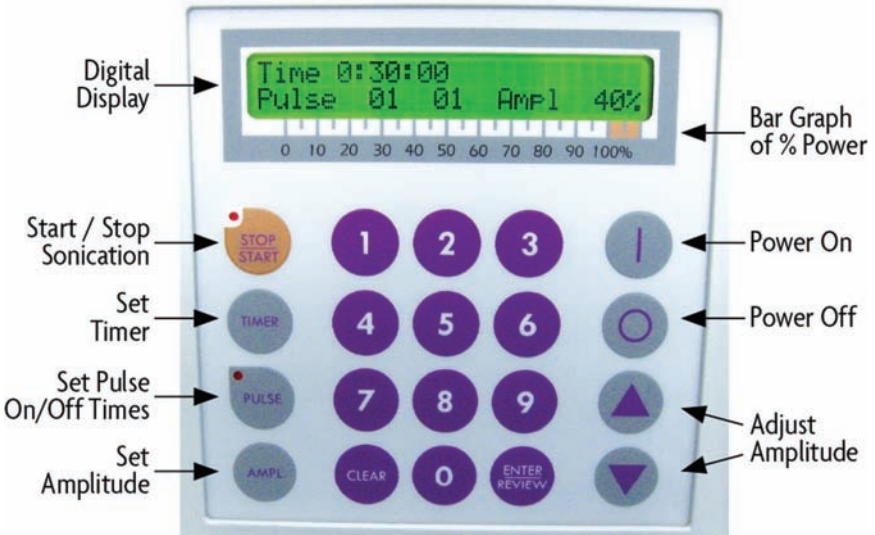
1. Place the rotator motor on the back of the water bath and hand tighten the white screws.
2. Screw the tube rack into the bottom of the rotator housing and place it onto the metal posts on top of the motor. To ensure the gears align and the housing is properly seated, you may need to rotate the rack forward or backward.
3. Loosen the thumbscrews on the back of the enclosure and open the door. Remove the sound proofing from the middle of the enclosure, then open the metal door at the bottom of the stand. (The doors are removed in these photos.)
4. Four connections will need to be made. With the rotator facing out, slide the water bath in slightly, then plug the Converter Cable on the bottom of the enclosure into the bottom of the converter. **IMPORTANT:** Plug the cable in all the way, or you may get an OVERLOAD error. Then, slide the entire unit in and place it on the stand.



5. Plug the Rotator Cable into the bottom of the rotator motor.
6. Attach the quick-connect fitting on the water tubing to those on the sides of the water bath.
7. Replace the panel of sound proofing back to the middle of the enclosure.
8. Make certain that the water bath / cup horn / converter is properly placed on the stand at the bottom of enclosure. Close the metal door at the bottom of the stand and tighten the thumbscrews securely, which will hold the unit in place in the enclosure.
9. Close the rear door and secure it by tightening the thumbscrews.
10. When you are ready for use, you can attach the rotator and converter cables from the generator and the water tubing from the thermoelectric chiller.



Functions of Keys, Controls, Indicators and Connectors



Front Panel	
LCD display	Displays prompts, as well as the following control parameters: <ul style="list-style-type: none"> • Amplitude selected. • Amount of output power delivered to the cup horn in watts, and as a percentage of total power. • Accumulated amount of energy in Joules delivered to the cup horn. • Set processing time and actual processing time • Elapsed time • Set pulsing cycle and actual pulsing cycle • Pulse duration
I key	Switches the main power on.
O key	Switches the main power off.
0 – 9 keys	Input digits.
START/STOP key	Starts or stops the sonicator. In STOP mode, the red indicator is off.
TIMER key	Used with the numeric keys to set the processing time.
PULSE key	Used with the numeric keys to set the duration of the ON and OFF pulses, which can be 1 to 59 seconds each. During the OFF portion of a cycle, the red indicator illuminates.
AMPL key	Controls the amplitude of vibration at the cup horn.
CLEAR key	Clears the preceding entry.
ENTER/REVIEW key	Used to enter and to review the various parameters.
▲ or ▼ key	Used with the AMPL key when the unit is on stand-by to set the amplitude of vibration at the probe tip. Also used to increase or decrease the amplitude in small increments while the unit is running. To accomplish this task, press the ENTER/REVIEW key twice to display AMPLITUDE CONTROL, then press the ▲ or ▼ key as required.

Operating Instructions

CAUTION

- **DO NOT** operate the power supply unless it is connected to the converter.
- **NEVER** allow liquid to spill into the converter.
- **NEVER** allow a cup horn to vibrate in air (not immersed in liquid) for more than 10 seconds.
- **AVOID** touching an activated cup horn with glass or plastic vials. It could crack or shatter the glass, or melt the plastic.
- **NEVER** place a washer between the converter and the cup horn, and do not apply grease to the mating surfaces or threads of the converter or cup horn.
- **NEVER** attempt to remove the cup horn by twisting the converter housing, as this may damage the electrical connections within the housing. Whenever you need to remove the cup horn from the converter, use the wrenches supplied.

KEY POINTS

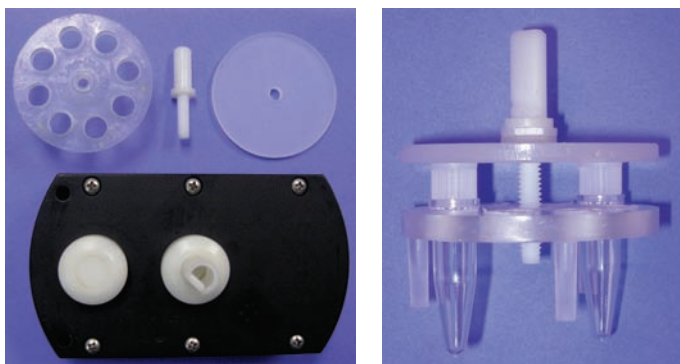
- Keeping the water level constant from experiment to experiment is important to achieve consistent results. You may wish to place some tape on the outside of the water bath to mark the water level so you can refill the same amount of water every time. The water level should be just above the Supply fitting at the top of the water bath.
- You may wish to keep water cold in the refrigerator to decrease the amount of time it takes to chill the water down to 3°C before you begin sonication. We do not recommend adding ice to the water bath as this can impact sonication results, and could potentially damage the chiller. It may take 30 minutes to chill the water completely, so put water in the water bath and begin chilling it first.
- Water should be drained from the water bath when the cup horn is not in use. We also recommend the use of an algicide (such as VWR Clear Bath Algicide, Cat. No. 13272-031) to prevent growth of algae in the water bath. Five to ten drops per bath are sufficient. Water with an algicide is the only acceptable liquid for use in the water bath.
- **DO NOT** use Methanol, Ethanol, or Isopropyl Alcohol as coolants, either by themselves or in water mixtures; use of these solutions can damage the chiller, and voids the chiller warranty.
- **DO NOT** use 25% propylene glycol or 25% ethylene glycol solutions to reduce the noise produced by the chiller; extended exposure will cause the acrylic water bath to turn cloudy.
- Degassed water transmits energy more efficiently. You may wish to degas the water before sonication. This can be done simply by running the sonicator for a few minutes.
- Polystyrene transfers sonic wave energy much more efficiently than polypropylene. For optimal results, use Active Motif's Polystyrene Sonication Tubes.
- After sonication, polystyrene tubes become brittle and may fail if they are spun at full speed in a desktop centrifuge. Either spin at a lower speed to collect the samples, or transfer them to fresh tubes (of any material) if your protocol uses a full-speed spin after sonication.

Set-up of the sonication equipment

1. If the converter has not yet been attached securely to the cup horn / water bath and placed in the sound enclosure, do this now. See pages 9-11 for instructions.
2. Add water to the water bath so the water level is just above the top of the upper fitting.
3. Connect the water tubes to the Supply and Return fittings on the back of the sound enclosure and to those on the back of the thermoelectric chiller. Be sure that each tube is attached to the same type of fitting (*i.e.* Supply to Supply and Return to Return).
4. Plug the DC power cord attached to the chiller's AC/DC power supply into the rear of the chiller. Plug the AC power cord into the chiller, and then into an electrical outlet.
5. Plug the power cord into the receptacle on the back of the generator, and then into an electrical outlet. If the unit is already on, press the **O** key to turn it off.
6. If the optional footswitch will be used, insert the plug into the jack on the rear panel.
7. Connect one end of the converter cable and the rotator cable to the appropriate plugs on the rear of the sound enclosure, then connect the other ends to the rear of the generator.

Placing sample(s) in the tube rack

The tube rack contains a cover and a post. The post has a snap fitting on one end and threads on the other. Be sure that the caps on your tubes are secured tightly to prevent water intrusion. Place your tubes in the tube rack, then put the threaded end of the post through the cover and screw it into the rack so the cover presses down on the tops of the tubes (right image). If only one sample is to be sonicated, you should place an empty tube opposite it so that the cover holds the tubes down straight. When assembled, push the post into the fitting on the bottom of the rotator.



Set-up of the chiller

Consult the manual that came with Active Motif's Thermoelectric Chiller for complete instructions. In short, after attaching the water tubing and the power cords as described above, simply turn the chiller on and wait for the display to light up. Use the UP and DOWN keys to change the temperature set point, then press ENTER. Active Motif recommends 3°C for shearing chromatin. To change the temperature set point, use the UP or DOWN keys followed by the ENTER key.

Important: As the block in the chiller will be colder than the fluid that is circulated, **DO NOT** set the temperature less than 3°C more than the fluid's freezing point (*i.e.* the set point for water should be $\geq 3^{\circ}\text{C}$). Otherwise, the block could freeze and crack.

Operation of the sonicator

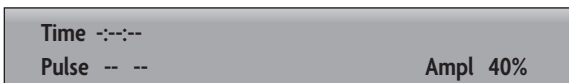
Press the **⏏** key to turn the unit on. The screen will display the power rating of the EpiShear Multi-Sample Sonicator and then the following control parameters:



AMPLITUDE: The desired amplitude must be set in order for the EpiShear Multi-Sample Sonicator to be operational. The other control parameters, Time and Pulse, do not have to be set for continuous operation. Pressing the **AMPL** key displays the amplitude selected, *e.g.* 40%. To set the amplitude at 40% when the EpiShear is not in operation, press the **AMPL** key and then use the numeric keys for a 40% reading on the screen, and then press the **ENTER/REVIEW** key.

Note: The amplitude may be set from 20% - 100%.

The screen will display:



Note: To clear an incorrect entry, press the **CLEAR** key.

1. The EpiShear Multi-Sample Sonicator is now ready for continuous operation. To energize the EpiShear, press the **START** key or the footswitch. The samples will rotate and the LED lights will turn on automatically whenever the unit is sonicating. To de-energize the EpiShear, press the **STOP** key or release the footswitch. If the Time or Pulse functions will be used, refer to the next sections below.

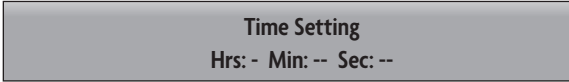
Note: The **START/STOP** key and footswitch are mutually exclusive. If the process is initiated by the **START** key, the footswitch becomes inoperative. If the process is initiated by the footswitch, the **STOP** key becomes inoperative.

2. To increase or decrease the amplitude in small increments when the EpiShear is in operation, press the **AMPL** key to display Amplitude Setting on the screen, then press the **▲** or **▼** key, as required.

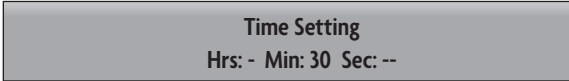
Because the amplitude required is application dependent and subject to the volume and composition of the sample, it is recommended that the amplitude be selected through experimentation, by increasing or decreasing the level of intensity as needed to properly process the sample to achieve desired results.

TIMER: If you are using the pulsed mode (below), the processing time will differ from the elapsed time because processing time monitors and controls only the ON portion of the duty cycle. For example, if the ON and OFF cycle are set for 30 seconds each and the processing time is set for 30 minutes, the total elapsed time will be 1 hour (*i.e.* 30 minutes ON and 30 minutes OFF).

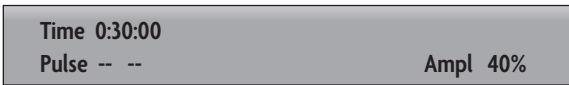
1. To set the processing time, press the **TIMER** key. The screen will display:



2. Using the numeric keys, set the processing time as required, for example:



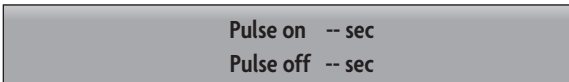
3. Press the **ENTER/REVIEW** key. The screen will display:



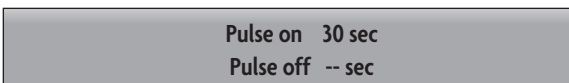
PULSER: The EpiShear can be set to pulse on and off, which prevents heat build up. The ON and OFF pulse durations can be set from 01 second to 59 seconds each. During the OFF portion of the cycle, the red indicator on the **PULSE** key will illuminate. If the OFF portion of the cycle exceeds three seconds, "Sonics in OFF Cycle" will display to warn that the unit is still in operation.

When the EpiShear Thermoelectric Chiller is used, it may not be necessary to set ON and OFF pulse cycles; the chiller alone can be sufficient to cool the samples. However, if total processing time will exceed 15 minutes or if the amplitude will be greater than 50%, use the pulse mode as the chiller may not be able to offset the heat being generated. While your settings will vary depending on your sample and application, typically we set the ON and OFF durations equally (*i.e.* 30 seconds ON and OFF). For higher amplitudes and/or longer sonication times, set the OFF time to be greater than the ON time (*i.e.* 15 seconds ON and 30-45 seconds OFF). Optimize these parameters for your specific needs. Keep in mind that while pulsing increases the total elapsed time, keeping the samples cold can only improve your results, so err on the side of more cooling.

1. To set the pulser, press the **PULSE** key. The screen will display:



2. Using the numeric keys, set the ON portion of the cycle from 01 second to 59 seconds, then press the **ENTER/REVIEW** key. The screen will display:



- Then, use the numeric keys and press **ENTER/REVIEW** to set the OFF portion of the cycle from 01 second to 59 seconds:

Pulse on 30 sec
Pulse off 30 sec

- Press the **ENTER/REVIEW** key. The screen will display all of the set parameters:

Time 0:30:00
Pulse 30 30 Ampl 40%

↑ ↓
On Cycle Off Cycle

- To energize the EpiShear, press the **START** key or the footswitch. The samples will rotate and the LED lights will turn on automatically whenever the unit is sonicating. To de-energize the EpiShear, press the **STOP** key or release the footswitch.

REVIEW: The REVIEW function provides a “window” on the process by displaying the various operating parameters without interrupting the process. Pressing the **ENTER/REVIEW** key repeatedly during processing will consecutively display the following information:

- Selected amplitude, *e.g.* Amplitude 40%
 - Selected processing time and elapsed processing time, *e.g.* Set 0:30:00 Time 0:22:10
 - Selected pulsing cycle and actual pulsing cycle, *e.g.* Pulse 01 01 / 01 00
 - Amount of power in watts, and accumulated amount of energy in JOULES delivered to the probe, *e.g.* 20 watts 0000000 Joules
- Note:** The amount of energy displayed will be for one cycle only. The initiation of a new cycle will reset the display to zero.
- Total elapsed time since processing was initiated, *e.g.* Elapsed time 0:44:20

Refer to the Appendix if the display indicates an **OVERLOAD** condition.

Sonication Shearing of Chromatin for ChIP

Active Motif offers a complete kit for preparing chromatin by sonication and then performing magnetic bead-based ChIP (ChIP-IT™ Express, Catalog No. 53008), as well as a kit just for preparing chromatin by sonication (ChIP-IT™ Express Shearing Kit, Catalog No. 53032). These kits contain all of the buffers needed, as well as optimized protocols. Below are some general guidelines.

Shearing tips

ChIP experiments usually require chromatin that has been sheared to 200-1500 bp. In general, shearing efficiency is improved through the use of a small shearing volume and a V-bottom tube rather than a round-bottom tube. Because the EpiShear Multi-Sample Sonicator uses an indirect

sonication method, use of polystyrene tubes will provide much better results than polypropylene tubes because polystyrene transfers sonic wave energy much more efficiently than polypropylene. However, as sonication can cause the polystyrene tubes to become brittle, do not centrifuge them at full speed after sonication. Use a lower speed or transfer the samples to fresh tubes (of any material) if your protocol requires a full-speed spin after sonication.

Optimizing the Shearing Conditions

Chromatin shearing conditions can vary significantly depending on the cell type and, occasionally the cell culture & cell stimulation conditions. However, after shearing has been optimized for a given cell type, those conditions usually give consistent results with that cell type. For this reason, we recommend that you determine the optimal shearing conditions the first time you make chromatin from a cell line by testing 3 different shearing conditions. This requires you to grow and work with three times the number of cells for optimization than you will need for subsequent ChIP experiments, where you will use your optimized conditions on a single plate of cells.

Suggested Starting Conditions

The optimal shearing conditions for your cell line should be determined empirically. As a general guideline, 7-10 minutes of sonication at 35-65% amplitude will work well for cell lines such as HeLa and 3T3 (Figure 3). If you are working with hard-to-shear samples such as lymphocytes, we have had success increasing the sonication time instead of the amplitude (*i.e.* 30 minutes at 50% amplitude). Again, you should test various parameters on your samples to determine what works best.

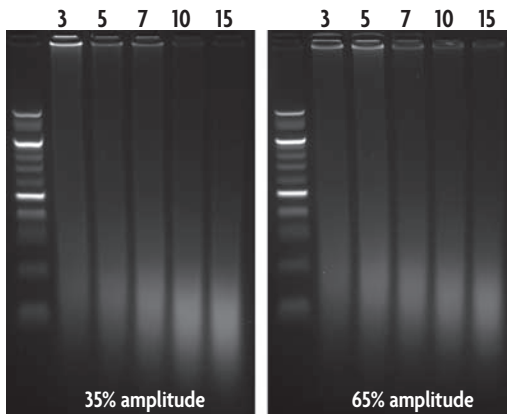


Figure 3: Chromatin ladder prepared by increasing the time and amplitude of sonication.

3T3 cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared for sonication using the reagents and protocol found in the ChIP-IT® Express Kit. 100 µl samples were then sonicated using the EpiShear Multi-Sample Sonicator at a 35% amplitude (left gel) or a 65% amplitude (right gel). From left to right on each gel, the samples were subjected to 3, 5, 7, 10 or 15 minutes of sonication. No pulsing was used.

The sonication performed at 35% amplitude demonstrates that by increasing the time of sonication you can shear the chromatin to your choice of size. Increasing the time of sonication has less of an impact on the samples sheared at 65% amplitude; the average size is somewhat consistent due to the increased power. However, a close examination shows that increased time reduces the amount of high-molecular weight material.

Appendix

Section A. Troubleshooting

Your EpiShear Multi-Sample Sonicator was designed to provide you with years of safe and dependable service. Nevertheless, because of component failure or improper usage, the possibility does exist that it might not perform as it should, shut down or stop working altogether. The most probable causes for malfunction are listed below and should be investigated.

- The converter / cup horn are not tightened together properly. Use the wrenches provided to ensure that this connection is adequately tightened.
- The Converter Cable in the sound enclosure is not completely plugged into the converter.
- A connector or cable is damaged.
- The unit was plugged into an electrical outlet that provides a different voltage from that required. See Electrical Requirements on page 8.
- The converter and/or cup horn have been dropped.
- The cup horn being used is damaged or worn past its useful life.
- A fuse(s) has failed.

Section B. Overload Condition

If the EpiShear Multi-Sample Sonicator stops working, and an OVERLOAD indication is displayed on the screen, check for possible causes as outlined in the above paragraph, then press the **O** key to turn the unit off, and then the **I** key to switch the unit back on.

If the problem persists after inspecting the above possibilities, contact Technical Services.

Section C. Maintenance

It is recommended to periodically inspect the unit, both visually and physically, to ensure optimum and safe performance. This inspection should be scheduled as a routine maintenance procedure, done with the unit power OFF and with the unit unplugged from the AC power source.

Long exposure to acids or caustics can result in corrosion of metal parts or components. Check the generator, converter and cables periodically for any signs of rust or discoloration. If discoloration is found, move the ultrasonic processor away from the source of the contamination.

Periodically examine the condition of the high-voltage cable that attaches from the generator to the back of the sound enclosure, as well as the high-voltage cable that is inside the sound enclosure that connects to the converter. Inspect the wire insulation for damage, such as wear, burning from hot plate contact or breakage from extended use or rough handling. Make certain the cables always have slack and are never tensioned. If necessary, move the generator or sound enclosure closer to one another to accomplish this.

WARNING: Do not use a cable with broken end connections, exposed wires or frayed insulation. High voltage is present in the cable; it is a shock hazard. **DO NOT** touch the converter assembly until the power switch is OFF and the unit is unplugged.

Cup Horn Maintenance

Sonicators create high-intensity vibrations that put stress on the converter and cup horn assembly. When sonicating, the sides and end of the cup horn must never be allowed to come in contact with anything but the water in the water bath.

Proper care of the cup horn is essential for dependable operation. The intense cavitation will, after usage for period of time, cause the cup horn to erode and the power output to decrease. The smoother and shinier the cup horn, the more power will be transmitted into the sample. The vibrations may also cause the cup horn tip to loosen from the converter over time or the threaded connection to accumulate debris.

Note: A loose cup horn will usually generate a loud piercing or squealing sound.

For that reason, it is recommended that a preventative maintenance schedule be adopted to examine the unit at regular intervals. The schedule should depend on frequency of use. Weekly maintenance schedules are recommended for units used frequently or monthly for those used infrequently. The cup horn must be examined for excessive wear and to ensure that the threaded connection is clean and attached properly to the converter. Use a cotton swab and alcohol (*i.e.* ethanol, isopropyl, *etc.*) to clean the threaded mating surfaces.

When excessive wear (corrosion/pitting of the cup horn end) is detected, the cup horn should be replaced with a new one.

WARNING: Never hand tighten the cup horn onto the converter; properly tighten them with the appropriate wrench set that was supplied with your unit.

Follow the steps below to detach the cup horn and converter, clean, then reattach them:

1. Use the wrench set provided with the unit to disconnect the cup horn from the converter.



2. Clean the threaded stud. Use alcohol and a cotton swab to remove any debris on the threading of the connecting stud. Allow the alcohol to dry completely.
3. Clean the threading in the converter. Use alcohol and a cotton swab to remove any debris on the threading. Do not allow any liquid to drip into the converter. Allow the alcohol to dry completely.
4. Reattach the cup horn to the converter. Screw the cup horn back onto the converter and tighten with the wrench set provided.



Note: You do not need to remove the cup horn / converter assembly from the water bath in order to detach the cup horn from the converter to inspect / clean the threaded connection between the cup horn and converter.

System Cleaning Instructions

The generator and converter may be cleaned using an acid-free cleaning solution (*i.e.* glass cleaner).

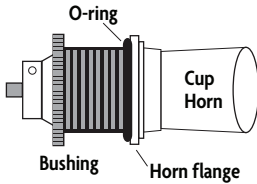
The cup horn should be cleaned using isopropyl alcohol. Cup horns are made from titanium and can be autoclaved (the converter is an electrical part and cannot be sterilized in this manner).

Periodically, the cup horn / water bath assembly should be taken apart so that the water bath can be cleaned with a mild detergent. This will also enable the cup horn to be cleaned more completely than is possible when it is attached to the water bath. The frequency of this will depend greatly on if an algicide is used regularly and if the water is drained from the water bath when the unit is not in use. The instructions on the following page illustrate how to assemble the cup horn / water bath. Simply reverse the steps to disassemble it for cleaning. Depending on how often this maintenance is performed, the use of a silicone grease on the O-rings is recommended as the repeated assembly and disassembly of the unit will eventually cause the O-rings to fit less tightly.

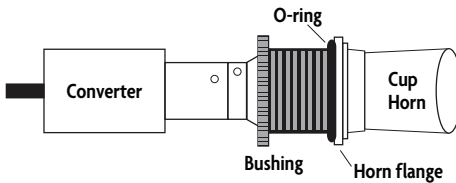
See page 7 for an exploded illustration that shows all of the parts in the order that they will be assembled.

Assembly / Disassembly of the Cup Horn / Water Bath

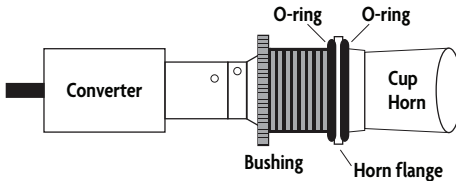
1. Using the provided wrenches, carefully remove the converter from the cup horn, as shown to the right.
2. Place one O-ring and the bushing on the side of the cup horn with the threaded stud.



3. Using the provided wrenches, tightly secure the cup horn back onto the converter.
IMPORTANT: Be certain the cup horn and converter are connected properly. If this connection is not sufficiently tightened, the generator may display an OVERLOAD error.

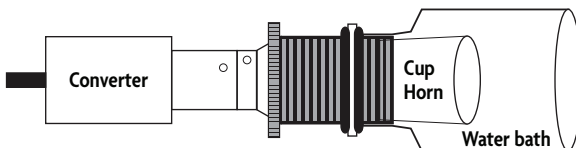


4. Place the 2nd O-ring on the other side of the horn flange.



Note: While it is not required, if you have silicone grease you may wish to lightly coat each O-ring, as this will extend their life and help ensure a good seal.

5. Place the water bath over the cup horn and secure it in place by screwing the bushing into the threaded portion of water bath, which will compress the two O-rings against the flange.



6. Fill the water bath / cup horn with water and check for any leakage. A properly assembled unit will not leak. Remove the water and proceed with the next step.

Section D. Limited Warranty

Your EpiShear Multi-Sample Sonicator (excluding the thermoelectric chiller) is warranted for a period of two years from the date of shipment against defects in material and workmanship under normal use as described in this instruction manual. The thermoelectric chiller is warranted against defects for a period of one year from the date of shipment.

During these warranty periods, Active Motif will, at its option, as the exclusive remedy, either repair or replace without charge for material and labor, the part(s) that prove to be defective, provided the unit is returned to us properly packed with all transportation charges prepaid.

Wear to the cup horn resulting from cavitation erosion is a normal consequence of ultrasonic processing, and is not covered by this warranty. All cup horns are manufactured to exacting specifications and are tuned to vibrate at a specific frequency. Using an out-of-tune cup horn will cause damage to the equipment and may result in warranty nullification. Active Motif assumes no responsibility for cup horns fabricated by another party or for consequential damages resulting from their use.

The above warranties do not apply to equipment or parts that have been subjected to unauthorized repair, modification, misuse, abuse, negligence or accident. Equipment or parts that show evidence of having been used in a manner inconsistent with their ordinary purpose or the operating instructions, or which have had the serial number altered or removed, will be ineligible for these warranties.

The aforementioned provisions do not extend the original warranty periods of any products that have either been repaired or replaced by Active Motif. All warranty claims must be received by Active Motif prior to expiration of the relevant warranty period.

ACTIVE MOTIF NEITHER ASSUMES NOR AUTHORIZES ANY PERSON TO ASSUME FOR IT ANY OTHER OBLIGATIONS OR LIABILITY IN CONNECTION WITH THE SALE OF ITS PRODUCTS. ACTIVE MOTIF HEREBY DISCLAIMS ALL REPRESENTATIONS AND WARRANTIES EXCEPT THE EXPRESS WARRANTIES ABOVE, INCLUDING ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. NO PERSON OR COMPANY IS AUTHORIZED TO CHANGE, MODIFY OR AMEND THE TERMS OF THIS WARRANTY IN ANY MANNER OR FASHION WHATSOEVER. UNDER NO CIRCUMSTANCES SHALL ACTIVE MOTIF BE LIABLE TO THE PURCHASER OR ANY OTHER PERSON FOR ANY INDIRECT, INCIDENTAL, CONSEQUENTIAL OR PUNITIVE DAMAGES, OR LOSS OF GOODWILL, PRODUCTION OR PROFIT IN CONNECTION WITH ITS PRODUCT, WHETHER IN CONTRACT, TORT OR OTHERWISE.

Section E. Service / Return of Equipment

An EpiShear Multi-Sample Sonicator in need of repair should be sent back to Active Motif. To do so, you must contact an Active Motif Customer Service Representative to obtain a Return Authorization Number (RA No.) before returning any instrument. Please have your Sales Order Number, date of purchase and the unit's serial number available when you call, or include it in your e-mail.

Care should be exercised to provide adequate packing to insure against possible damage in shipment. The unit should be sent to the "Service Department" of your nearest Active Motif office, with all transportation charges prepaid and return of shipment address indicated.

Important

Any item(s) being returned must include a signed Safety Certification Form (below) to certify that the EpiShear Multi-Sample Sonicator and/or the accessories being returned for repair are free of any biohazardous or radioactive material, and are safe for handling.

Do not return any equipment without an RA No. and a completed Safety Certification Form. Active Motif will send back any equipment it receives that has not been certified to be safe.

Section F. Safety Certification Form

Items being returned: _____

Please check only one item below:

- The equipment was never used or exposed to any radiological, biological or chemical agents and is safe to handle, use or dispose of.
- The equipment was used, but not in conjunction with or exposed to any radiological, geological or chemical agents and is safe to handle, use or dispose of.
- The equipment was used in conjunction with or exposed to radiological, biological or chemical agents and has been decontaminated, rendering it safer for handling, use or disposal.

Authorization

By accepting authorization to return the equipment listed above, the undersigned assumes all responsibility and liability for radiological, biological and chemical decontamination. Delivery of the equipment can be refused if necessary documentation is not provided or where it is determined that the equipment has not been properly decontaminated. If it is determined that the equipment was not properly decontaminated, the Authorized Repair Facility reserves the right to bill the customer for any and all costs associated with the decontamination and/or appropriate disposal of the equipment. In the event the equipment has been exposed to radiological contamination, the signature of the Radioactive Safety Officer is required.

Printed Name: _____

RA No: _____

Signature _____

Date: _____

Section G. Related Products

Sonication	Format	Catalog No.
EpiShear™ Multi-Sample Sonicator / Chiller	110V 230V	53062 53063
EpiShear™ Multi-Sample Sonicator	110V 230V	53065 53066
EpiShear™ Thermoelectric Chiller	100V-240V	53068
EpiShear™ Probe Sonicator	110V 230V	53051 53052
EpiShear™ 5/64" (2 mm) Sonicator Probe	1 probe	53056
EpiShear™ 1/8" (3.2 mm) Sonicator Probe	1 probe	53053
EpiShear™ 1/4" (6.4 mm) Sonicator Probe	1 probe	53057
EpiShear™ Cooled Sonication Platform, 1.5 ml	1 platform	53073
Support Stand / Converter Clamp	1 unit	53054
Sound Enclosure with Converter Clamp	1 enclosure	53055
Large Sound Enclosure	1 unit	53060
EpiShear™ Replacement Converter	1 unit	53058
Footswitch	1 unit	53059

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express Shearing Kit	10 rxns	53032
ChIP-IT™ Express Enzymatic Shearing Kit	10 rxns	53035
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
RNA ChIP-IT™	25 rxns	53024
Chromatin IP DNA Purification Kit	50 rxns	58002
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Siliconized Tubes, 1.7 ml	25 tubes	53036
ChIP-IT™ Control qPCR Kit – Human	5 rxns	53026
ChIP-IT™ Control Kit qPCR – Mouse	5 rxns	53027
ChIP-IT™ Control Kit qPCR – Rat	5 rxns	53028
RNA ChIP-IT™ Control Kit – Human	25 rxns	53024
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
Bridging Antibody for Mouse IgG	500 µg	53017

ChIP-validated Antibodies

For an up-to-date list of over 150 ChIP-validated antibodies, please visit www.activemotif.com/chipabs.

ChIP Control qPCR Primer Sets

For an up-to-date list of over 30 qPCR Primer Sets, please visit www.activemotif.com/chipprimers.

Whole Genome Amplification	Format	Catalog No.
GenoMatrix™ Whole Genome Amplification Kit	1 kit	58001

Modified Histones Array	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001

Histone ELISAs	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 acetyl Lys9 ELISA	1 x 96 rxns	53114
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 acetyl Lys14 ELISA	1 x 96 rxns	53115
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110

Histone Purification & Chromatin Assembly	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	10 rxns	40026
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Recombinant Methylated, Acetylated and Phosphorylated Histone Proteins

For an up-to-date list of Recombinant Histone Proteins, please visit www.activemotif.com/recomphis.

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Histone Demethylase Activity	Format	Catalog No.
Histone Demethylase Assay (Fluorescent)	48 rxns	53200

DNA Methylation	Format	Catalog No.
hMeDIP	10 rxns	55010
Hydroxymethyl Collector™	25 rxns	55013
MeDIP	10 rxns	55009
MethylDetector™	50 rxns	55001
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
DNMT Activity / Inhibition Assay	96 rxns	55006
Methylated DNA Standard Kit	3 x 2.5 µg	55008
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America

1914 Palomar Oaks Way, Suite 150

Carlsbad, CA 92008

USA

Toll Free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

E-mail: tech_service@activemotif.com

Active Motif Europe

Avenue Reine Astrid, 92

B-1330 La Hulpe, Belgium

UK Free Phone: 0800 169 31 47

France Free Phone: 0800 90 99 79

Germany Free Phone: 0800 181 99 10

Telephone: +32 (0)2 653 0001

Fax: +32 (0)2 653 0050

E-mail: eurotech@activemotif.com

Active Motif Japan

Azuma Bldg, 7th Floor

2-21 Ageba-Cho, Shinjuku-Ku

Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638

Fax: +81 3 5261 8733

E-mail: japantech@activemotif.com

Visit Active Motif on the worldwide web at <http://www.activemotif.com>

At this site:

- Read about who we are, where we are, and what we do
- Review data supporting our products and the latest updates
- Enter your name into our mailing list to receive our catalog, *MotifVations* newsletter and notification of our upcoming products
- Share your ideas and results with us
- View our job opportunities

Don't forget to bookmark our site for easy reference!