

EpiShear™ Probe Sonicator

(version A6)

Catalog Nos. 53051 (110V) & 53052 (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 2014 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.

© 2014 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 |
| Specifications | 3 |
| Principles of Operation | 4 |
| Included Components | 6 |
| EpiShear™ Accessories | 7 |
| Functions of Keys, Controls, Indicators, and Connectors | 8 |
| Preparation for Use | 10 |
| Operating Instructions | 11 |
| Sonication Shearing of Chromatin for ChIP | 14 |
| Appendix | |
| Section A. Troubleshooting | 15 |
| Section B. Overload Condition | 15 |
| Section C. Maintenance | 16 |
| Section D. Limited Warranty | 18 |
| Section E. Service / Return of Equipment | 19 |
| Section F. Safety Certification Form | 19 |
| Section G. Related Products | 20 |
| Technical Services | 22 |

Overview

Active Motif's EpiShear™ Probe Sonicator is ideal for preparing sheared chromatin for shearing chromatin or DNA from small or large sample sizes for use in chromatin immunoprecipitation (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 is a microprocessor-based, ultrasonic processor that offers both programmable and manual operation. A key feature is the ability to program a pulse mode that can be used to prevent heat build up in temperature-sensitive samples. In addition, pulsing enhances processing by allowing the material to settle back under the probe after each burst.

The unit has a digital display that makes 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. The display also shows the total elapsed time, and provides real-time energy monitoring of both wattage and joules.

The EpiShear is supplied with an ultrasonic electric generator, a piezoelectric converter, a 1/8" microtip probe, converter & power cables, a wrench set and a comprehensive manual. It is backed by a two-year warranty.

A variety of accessories are available, including different sizes of microtip probes, a converter stand for hands-free use, a sound enclosure to reduce noise and a footswitch that makes it more convenient to deliver short pulses when using the unit in manual mode.

| product | format | catalog no. |
|---------------------------|---------------|--------------------|
| EpiShear™ Probe Sonicator | 110V 230V | 53051 53052 |

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 and/or property damage. For total safety and equipment protection, read the 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 cable. 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.
- Hearing protection is highly recommended. Use a sound-abating enclosure or ear protection.
- **NEVER** immerse the converter in liquids of any kind, or allow condensed moisture or liquid to drip into the converter.
- **NEVER** touch the tip of a vibrating probe. It can cause severe burns and tissue damage.
- **NEVER** allow a microtip to vibrate in air (not immersed in liquid) for more than 10 seconds.
- **NEVER** hold or clamp the converter by the front driver or by the probe itself. This can cause permanent damage to the system. Support the converter only by clamping around the upper portion of the converter housing.
- **DO NOT** allow the tip of a vibrating probe to touch the counter or any other hard surface. It could damage the probe, overload the generator, and/or damage the surface.
- Avoid touching the bottom or sides of glass or plastic containers with an activated probe. 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 cable 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. Stop sonication through the touch screen prior to turning off the AC power.

Specifications

| Generator | 110V (Catalog No. 53051) | 230V (Catalog No. 53052) |
|------------------|------------------------------------------------------|------------------------------|
| Input Voltage | 100 VAC – 120 VAC @ 50/60 Hz | 220 VAC – 240 VAC @ 50/60 Hz |
| Rated Current | 2.4 Amps max. | 1.2 Amps max. |
| Fuse Rating | 3 Amps (Slo-blow)* | 1.6 Amps (Slow-blow)* |
| Weight | 7 lbs. (3.2 Kg) | |
| Dimensions | 8" W x 13.75" L x 5.75" H (203 mm x 349 mm x 146 mm) | |
| Output Voltage | 1000 V rms (max.) | |
| Output Frequency | 20 KHz | |

| Converter (also sold separately as Catalog No. 53058) | |
|-------------------------------------------------------|--------------------------------------|
| Weight | 0.75 lbs. (0.34 Kg) |
| Dimensions | 6" L x 1.25" Diameter (15 cm x 3 cm) |
| Materials | Aluminum Alloy |

| 1/8" Microtip (also sold separately as Catalog No. 53053) | |
|-----------------------------------------------------------|-------------------------------------------|
| Weight | 0.25 lbs. (0.11 Kg) |
| Dimensions | 5.4" L x 0.5" Diameter (13.8 cm x 1.3 cm) |
| Materials | Titanium Alloy |

| 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 potential 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.

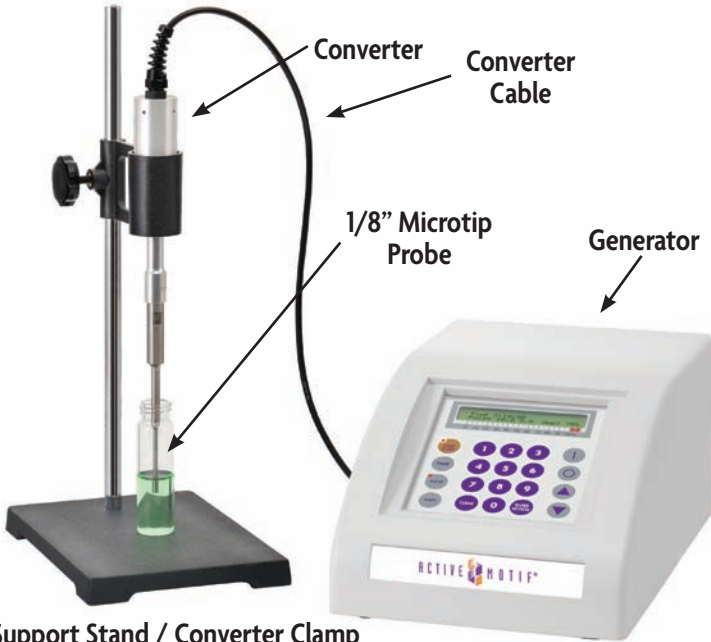
The AMPLITUDE control enables the ultrasonic vibrations at the probe tip to be set to any desired level. Although the degree of cavitation/ultrasonic energy required to process the sample can readily be determined by visual observation, the amount of power required cannot be pre-determined. A sensing 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 (120 watts) that the EpiShear Probe 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 Probe Sonicator is supplied with an ultrasonic electric generator, a piezoelectric converter, a 1/8" tip sonicator probe, converter & power cables and a wrench set. See Accessories for information on ordering additional probes, a replacement converter or stands and sound enclosures.



Support Stand / Converter Clamp
(not included; sold separately as Cat. No. 53054)

| Technical Specifications | |
|------------------------------------------|--------------------------------------------------------------------|
| Sample size: (dependent on the probe) | 200 µl - 50 ml (500 µl - 15 ml with the included 1/8" probe) |
| Power Rating: | 120 watts |
| Frequency: | 20 kHz |
| Programmable Timer: | 1 second to 10 hours |
| Adjustable Pulse: | 1 second to 59 seconds |
| Adjustable Amplitude: | 20% - 100% |
| Dimensions: | 8" W x 13.75" L x 5.75" H (203 mm x 349 mm x 146 mm) |
| Voltage: | 110V @ 50/60 Hz or 230V @ 50/60 Hz |

EpiShear™ Accessories



Microtip Probes

| | | | |
|-----------------------------------------|-----------------------|------------------|--------------------|
| Processing Volume: | 200 µl - 5 ml | 500 µl - 15 ml | 10 ml - 50 ml |
| Tip Diameter: | 5/64" (2 mm) | 1/8" (3.2 mm) | 1/4" (6.4 mm) |
| Amplitude: (Intensity Level) | 200 µm (very high) | 180 µm (high) | 120 µm (medium) |
| Catalog No.: | 53056 | 53053 | 53057 |



Support Stand / Converter Clamp
Catalog No. 53054



EpiShear™ Replacement Converter
Catalog No. 53058

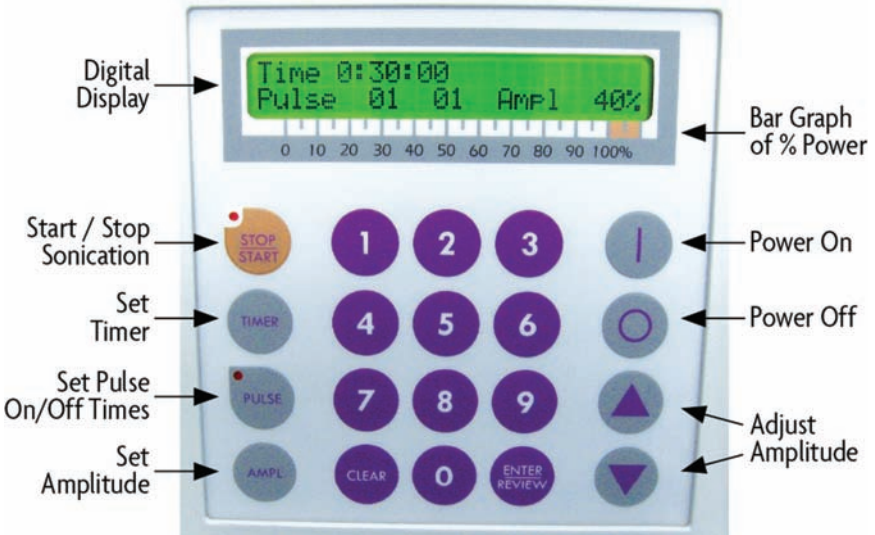


EpiShear™ Cooled Sonication Platform
Catalog Nos. 53080, 53081 & 53082
Exterior Dimensions: 28" H x 14.25" W x 12" D
(711 mm x 362 mm x 305 mm)

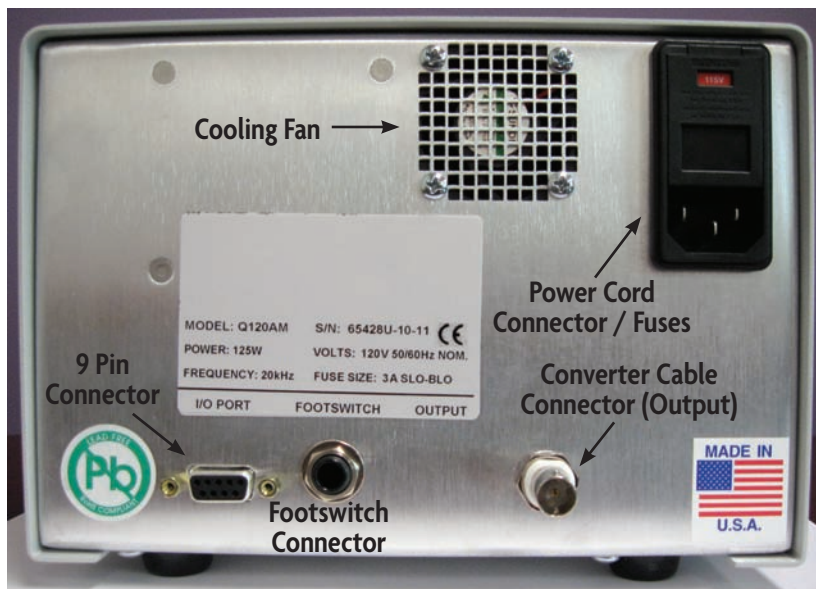


Footswitch
Catalog No. 53059

Functions of Keys, Controls, Indicators, and Connectors



| Front Panel | |
|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| LCD display | <p>Displays prompts, as well as the following control parameters:</p> <ul style="list-style-type: none"> • Amplitude selected. • Amount of output power delivered to the probe in watts, and as a percentage of total power. • Accumulated amount of energy in Joules delivered to the probe. • 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 the STOP mode, the red indicator goes 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 tip of the probe. |
| 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. |



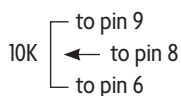
Rear Panel

| | |
|-------------------------------------------|--------------------------------------------------------------------------------|
| 9 pin D-sub Connector (I/O Port) | Connects to external actuation device; enables power and frequency monitoring. |
| Footswitch Connector | Connects to the footswitch cable. |
| Converter Cable Connector (Output) | Connects to the converter. |
| Power Supply Connector | Connects to the electrical line cord and encases the fuse(s). |

9 pin D-sub Connector

| | |
|-------------------------|------------------------------------------------------------------------------------|
| Pin No. 1 | Not connected |
| Pin No. 2 | Not connected |
| Pin No. 3 | Not connected |
| Pin No. 4 | Enables connection to a frequency counter. |
| Pin No. 5 | Enables connection to an external power monitor (5 mv = 1 watt) |
| Pin No. 6 | Ground |
| Pin No. 7 | Energizes the EpiShear when connected to ground. |
| Pin Nos. 8 and 9 | Enables the intensity to be remotely adjusted using an external 10k potentiometer. |

Note: To vary the intensity remotely using a variable DC power supply (0-5V) instead of a 10K potentiometer, connect positive to pin 8 and negative to pin 6.



Preparation for Use

Inspection

Prior to installing the EpiShear Probe 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 Probe 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 Probe Sonicator requires a fused, single phase 3-terminal grounding type electrical outlet capable of supplying 50/60 Hz at 100 volts, 115 volts, 220 volts, or 240 volts, depending on the voltage option selected. For power requirements, check the label on the back of the unit.

Should you ever need to convert the unit for different voltage operation, proceed as follows.

1. Ensure that the power cord is not connected to the electrical outlet.
2. Open the fuse holder cover using a small screwdriver.
3. Pull out the red fuse holder from its housing.
4. To convert from 100/115V to 220/240V, replace the two 3 Amp slow blow fuses, with 1.6 Amp fuses.
5. To convert from 220/240V to 100/115V, reverse the procedure above.
6. Rotate the fuse holder 180° from its original position, and reinsert it into its housing. For 100/115V operation the voltage displayed should be 115. For 220/240V operation the voltage displayed should be 220.
7. Change the electrical power cord as required.



**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 Probe 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.

When positioning the unit, be sure to leave adequate space behind the unit so that all connections can be easily disconnected.

Operating Instructions

CAUTION

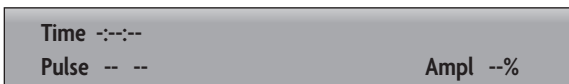
- **DO NOT** operate the power supply unless it is connected to the converter.
- **NEVER** allow liquid to spill into the converter.
- **NEVER** allow a microtip to vibrate in air (not immersed in liquid) for more than 10 seconds.
- **DO NOT** allow the vibrating microtip to contact anything but the sample.
- **NEVER** place a washer between the probe and the converter.
- **NEVER** apply grease to the mating surfaces or threads of the converter, probe or microtip.
- Should it become necessary to remove a probe, use the wrenches supplied. **NEVER** attempt to remove the probe by twisting the converter housing, as this may damage the electrical connections within the housing.

Set-up

1. Plug the power cord into the receptacle on the back of the power supply.
2. Plug the power cord into the electrical outlet. If the unit is already on, press the **O** key to turn it off.
3. If the optional footswitch will be used, insert the plug into the jack on the rear panel.
4. If the converter and probe assembly are not already assembled, screw the probe onto the converter; use the wrenches provided to tighten it securely. (See instructions in the Maintenance section for attaching and detaching microtips).
5. If using a laboratory stand, mount the converter/probe assembly using a clamp. Be sure to secure the clamp to the upper section of the converter housing only. Do not secure the clamp to any other portion of the converter/probe assembly.
6. Connect the converter cable to the power supply.

Operation

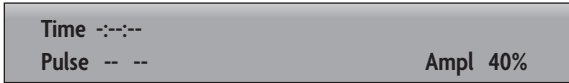
Press the **I** key to turn the unit on. The screen will display the power rating of the EpiShear Sonicator and the following control parameters:



AMPLITUDE: The desired amplitude must be set in order for the EpiShear Probe Sonicator to be operational. The other control parameters, Time and Pulse, do not have to be set for continuous operation. **AMPL** 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. Immerse the microtip half way into the sample. If the probe is immersed to an insufficient depth, air will be injected into the sample, causing the sample to foam. Also, ensure that the probe tip is not touching the wall of the sample vessel; it will not vibrate properly, and may be damaged.
2. The EpiShear Probe Sonicator is now ready for continuous operation. To energize the EpiShear, press the **START** key or the footswitch. 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 appropriate paragraphs below.

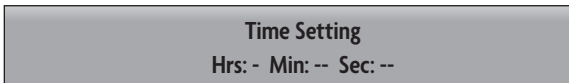
Note: The **START/STOP** key and footswitch are mutually exclusive. If the process is initiated by the **START** key, the footswitch becomes inoperative. If the process is initiated by the footswitch, the **STOP** key becomes inoperative.

3. 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.

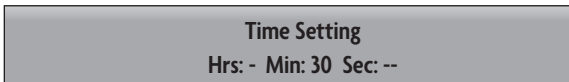
Because the amplitude required is application dependent and subject to the volume and composition of the sample, it is recommended that the amplitude be selected through experimentation, by increasing or decreasing the level of intensity as needed to properly process the sample to achieve desired results.

TIMER: In the pulsed mode, the processing time will differ from elapsed time because processing time monitors and controls only the ON portion of the duty cycle. For example, for 1 hour of processing time, elapsed time will be 2 hours if the ON and OFF cycle are each set for 1 second.

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



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

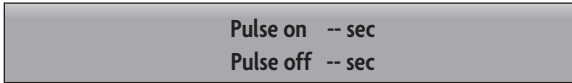


3. Press the **ENTER/REVIEW** key. The screen will display:

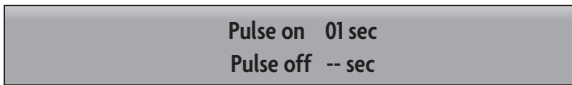


PULSER: Because sonicators generate heat, pulsing the EpiShear on and off helps to prevent heat build up in temperature-sensitive samples. In addition, pulsing enhances processing by allowing the material to settle back under the probe after each burst. The ON and OFF pulse durations can be set independently from 01 second to 59 seconds. 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, a cautionary message - Sonics in OFF Cycle - will warn the operator against touching the probe.

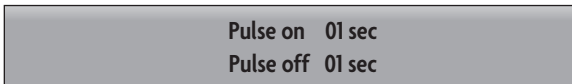
- To set the pulser, press the **PULSE** key. The screen will display:



- Using the number keys, set the ON portion of the cycle, then press the **ENTER/REVIEW** key. The screen will display:



- Then, use the number keys and press **ENTER/REVIEW** to set the OFF portion of the cycle:



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



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. Initiating 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 complete kits for preparing chromatin by sonication and then performing magnetic bead-based ChIP (ChIP-IT™ Express, Catalog No. 53008), or just preparing the chromatin (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 from those kits. As the EpiShear was used to develop our kits and protocols, we recommend using ChIP-IT Kits with the EpiShear.

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. Also, note that shearing is inefficient if the chromatin sample becomes emulsified with air bubbles. This can be avoided by using lower shearing power and by turning the power up gradually. If a chromatin preparation becomes emulsified inadvertently, discontinue shearing and centrifuge the sample for 4 minutes at 8,000 rpm at 4°C in a microcentrifuge to remove trapped air. Finally, to prevent overheating and denaturation of chromatin, samples should be kept on ice as much as possible during shearing, and shearing should be performed discontinuously (see Figure 1 below). If possible, shear in a chiller designed to keep samples cold during 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.

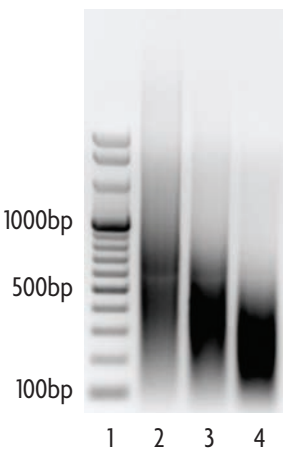


Figure 1: Gel analysis of HeLa chromatin following sonication.

HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the ChIP-IT Express Kit. Three samples of approximately 300 μ l each were sheared with 5, 10 and 20 pulses at 25% amplitude using the EpiShear with a 1/8" probe. Each pulse consisted of a 20-second sonication followed by a 30-second rest on ice to prevent heat build up. The sheared chromatin samples were subjected to cross-link reversal, treated with Proteinase K and RNase A, then phenol/chloroform extracted and precipitated as described in the ChIP-IT Express manual. Samples were separated by electrophoresis through a 1% agarose gel. Optimally sheared chromatin will yield a smear between 200-1500 bp.

Lane 1: 100 to 1000 bp ladder.

Lane 2: HeLa DNA sheared for 5 pulses (optimal).

Lane 3: HeLa DNA sheared for 10 pulses (optimal).

Lane 4: HeLa DNA sheared for 20 pulses (over-sheared).

Note: From this experiment, the samples sonicated for both 5 and 10 pulses are suitable for use in ChIP.

Appendix

Section A. Troubleshooting

Your EpiShear Probe 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.

- 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 10.
- The microtip is not tightened properly with the wrenches provided.
- The converter and/or microtip have been dropped.
- The microtip being used is damaged or worn past its useful life.
- A fuse(s) has failed. If a fuse(s) has failed, proceed as follows:
 1. Turn the unit off by pressing the **O** key, then disconnect the power cord from the electrical outlet.
 2. Open the fuse holder cover using a small screwdriver, and pull out the red fuse holder from its housing.
 3. Replace the fuse(s).
 4. Reconnect the line cord to the electrical outlet, press the **I** key to turn the unit on, then set the amplitude to 100. With the probe in air (out of the sample), the wattmeter should read below 10 watts. If the reading exceeds 10 watts, press the **O** key to turn the unit off, then disconnect the probe from the converter.
 5. Press the **I** key to turn the unit on. If the wattmeter reads below 10 watts, the probe has failed or is out of tune due to excessive erosion, and should be replaced. If the wattmeter reads above 20 watts, either the converter or power supply has failed and the complete EpiShear Probe Sonicator, and all components, should be returned for repair.

Section B. Overload Condition

If the EpiShear Probe 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 all of the above possibilities, please contact Customer Service for additional assistance, or to replace a worn microtip or damaged part.

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 contaminant.

Examine the condition of the high voltage cable that attaches the converter to the generator. Inspect the wire insulation for damage, such as wear, burning from hot plate contact or breakage from extended use or rough handling. In general use, the cable assembly should not be used to carry the converter or pull it toward the user. Make certain the cable always has slack and is never tensioned. If necessary, move the generator or converter assembly 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.

Microtip/Probe Maintenance

Sonicators create high-intensity vibrations that put stress on the converter and probe assembly. The sides and end of the probe must never be allowed to come in contact with anything but the solution. When using a microtip, the stress resulting at the point of contact with the vessel could cause the microtip to fracture.

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

Note: A loose probe 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 tip 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 probe tip) is detected, the probe should be replaced with a new one.

WARNING: Never hand tighten probes or horns onto the converter; properly tighten them with the appropriate Wrench Set.

Follow the steps below for attaching and detaching microtip probes:

1. Disconnect the probe from the converter. Use the wrench set provided with the unit.



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 probe to converter. Screw the probe back onto the converter and tighten with the wrench set provided.



System Cleaning Instructions

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

Probes should be cleaned using isopropyl alcohol. Probes are made from titanium and can be autoclaved (the converter is an electrical part and cannot be sterilized in this manner). Before each procedure, place the probe tip in water or alcohol and turn the power on for a few seconds to remove residue. The tip also can be sterilized using alcohol with the power on.

Section D. Limited Warranty

Your EpiShear Probe Sonicator (excluding ultrasonic probe(s)) 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. During the warranty period, 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.

Ultrasonic probes are guaranteed against defects for a period of one year from the date of shipment. A defective probe will be replaced one time without charge, if failure occurs within the warranty period. Wear resulting from cavitation erosion is a normal consequence of ultrasonic processing, and is not covered by this warranty.

All probes are manufactured to exacting specifications and are tuned to vibrate at a specific frequency. Using an out-of-tune probe will cause damage to the equipment and may result in warranty nullification. Active Motif assumes no responsibility for probes 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 this warranty.

The aforementioned provisions do not extend the original warranty period of any product that has either been repaired or replaced by Active Motif. All warranty claims must be received by Active Motif prior to expiration of the 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 Probe 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 EpiShear Sonicator 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 Probe 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 | 53062 |
| | 230V, EU | 53063 |
| | 230V, UK | 53064 |
| EpiShear™ Multi-Sample Sonicator | 110V | 53065 |
| | 230V | 53066 |
| | 230V, UK | 53067 |
| EpiShear Thermoelectric Chiller | 110V | 53068 |
| | 230V, EU | 53069 |
| | 230V, UK | 53070 |
| EpiShear™ Probe Sonicator | 110V | 53051 |
| | 230V | 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 Sonicator Platform, 1.5 ml | 1 platform | 53073 |
| Support Stand / Converter Clamp | 1 unit | 53054 |
| Sound Enclosure with Converter Clamp | 1 enclosure | 53055 |
| 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 Kit – Human | 5 rxns | 53010 |
| ChIP-IT™ Control Kit – Mouse | 5 rxns | 53011 |
| ChIP-IT™ Control Kit – Rat | 5 rxns | 53012 |
| 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 125 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/recombhis.

| 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™ | 25 rxns | 55002 |
| 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!