

Autotune Series High Intensity Ultrasonic Processor

**70 Watt Model • 100 Watt Model
130 Watt Model • 150 Watt Model**

USER'S GUIDE

RETURN OF EQUIPMENT

It is suggested that an Ultrasonic Processor in need of repair be sent back to the factory.

In order to receive prompt service, always contact the factory before returning any instrument. Include date of purchase, model number and serial number. For instruments not covered by the warranty, a purchase order should be forwarded to avoid unnecessary delay. Care should be exercised to provide adequate packing to insure against possible damage in shipment. The Ultrasonic Processor should be sent to the "Service Department" with all transportation charges prepaid and return of shipment indicated.

Please obtain a *Return Authorization Number* prior to returning the instrument.

IMPORTANT

I CERTIFY THAT THE ULTRASONIC PROCESSOR AND/OR ACCESSORIES RETURNED FOR REPAIR ARE FREE OF ANY BIOHAZARDOUS OR RADIOACTIVE MATERIAL AND ARE SAFE FOR HANDLING. DO NOT RETURN ANY EQUIPMENT UNLESS SUCH CERTIFICATION CAN BE MADE.

IMPORTANT SAFEGUARDS

READ BEFORE INSTALLING OR USING THE EQUIPMENT

Your Ultrasonic Processor has been designed to assure maximum operator safety. However, no design can completely protect against improper usage, which may result in bodily injury and/or property damage. For your protection and equipment safeguard, observe the following warnings at all times, read the operating instructions carefully before you attempt to operate the equipment, and retain this instruction manual for future reference.

- High voltage is present in the power supply. Never remove the cover unless qualified to do so.
- To avoid electrical shock, disconnect electrical power cord before removing cover for servicing.
- Make sure the Ultrasonic Processor is properly grounded with a 3-prong plug. Before plugging in, test electrical outlet for proper earth grounding.
- Never operate the power supply unless it is connected to the converter.
- Never operate a probe with replaceable tip without a tip.
- Never touch a vibrating probe.
- It is recommended that ear protection be used when operating the Ultrasonic Processor.

The Ultrasonic Processor supplied with this instruction manual is constructed of the finest material and the workmanship meets the highest manufacturing standards. It has been thoroughly tested and inspected before leaving the factory and when used in accordance with the procedures outlined in this manual, will provide you with many years of safe and dependable service.

SECTION III - SERVICE INFORMATION

OVERLOAD CONDITION

The fuse(s) protect the Ultrasonic Processor against improper usage, or malfunction. Should a fuse fail, proceed as follows:

1. Ensure that the probe and/or tip is securely fastened.
2. Replace the fuse.
3. Set the AMPLITUDE control to 100 . With the 70 and 100 watt mode, set the PULSE/CONTINUOUS switch to CONTINUOUS. With the probe in air (out of the sample), the wattmeter should read below 20 watts. If the reading exceeds 20 watts, set the AMPLITUDE control to OFF, and disconnect the probe from the converter.
4. Set the AMPLITUDE control to 100 . 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 10 watts, either the converter or power supply has failed and should be returned for service.

LOW SURFACE TENSION LIQUIDS - ORGANIC SOLVENTS

All probes, including those with replaceable tips, are tuned to resonate at a specific frequency. If the replaceable tip is removed or isolated from the rest of the probe, that element will no longer resonate at that frequency, and the power supply will fail. Low surface tension liquids penetrate the interface between the probe and the replaceable tip, and carry the particulates into the threaded section, isolating the tip from the probe. When processing low surface tension liquids with a 13mm (1/2") probe, ALWAYS use a solid probe.

Before each application, place the probe tip in water or alcohol and energize the power supply for a few seconds to remove any residue.

If concerned with sample loss in test tube due to sticking, siliconize the test tube as follows: Wash and dry the test tube thoroughly, coat with silicone, then air-dry. "Sigmacote" manufactured by Sigma Chemical Co., 3050 Spruce Street, St. Louis, Missouri 63103, U.S.A., phone (314) 771-5765, is ideally suited for that purpose.

Probes may be autoclaved, or sterilized either by immersing in boiling water or in a detergent bactericide and a disinfectant.

High viscosity and concentration are problematic. 5,000 cps and 15% concentration by weight are maximum limits. If the sample is so thick that it will not pour or circulate easily, it is too thick and cannot be processed with ultrasonics.

SECTION I – INSTALLATION

INSPECTION

Prior to installing the Ultrasonic Processor, perform a visual inspection to detect any evidence of damage which might have occurred during shipment. Before disposing of any packaging material, check it carefully for small items.

The Ultrasonic Processor was carefully packed and thoroughly inspected before leaving our factory. Responsibility for its safe delivery was assumed by the carrier upon acceptance of the shipment. Claims for loss or damage sustained in transit must, therefore, be made upon the carrier.

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

ELECTRICAL REQUIREMENTS

The Ultrasonic Processor 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 instrument.

Prior to plugging the electrical cord into the wall outlet, ensure that the power module on the back of the instrument displays the proper voltage. If not, disconnect the power cord, and using a screwdriver open the power module, remove the fuse holder, rotate it and reinsert it. For 100 and 115 volts, the voltage displayed will be 115. For 220 and 240 volts, the voltage displayed will be 220.

WARNING

For your personal safety, the electrical line cord is equipped with a 3-prong grounding plug. Do not, under any circumstances, defeat the grounding feature of the power cord by removing the grounding prong. The plug must be plugged into a mating 3-prong grounding type wall receptacle.

INSTALLING THE ULTRASONIC PROCESSOR

The Ultrasonic Processor should be installed in an area that is free from excessive dust, dirt, explosive and corrosive fumes, and extremes of temperature and humidity.

processing time, using a narrower vessel, and lowering the temperature of the liquid will usually prevent aerosoling and foaming. Do not use antifoaming agents or surfacants.

During cavitation, free radicals are formed which, if they are allowed to accumulate, can greatly affect the biological integrity of the sample by reacting with proteins, polysaccharides, or nucleic acids. Although during short periods of processing their formation is not normally considered a problem; for prolonged processing, the addition of free radical scavengers such as N₂O, cysteine, reduced glutathione, dithiothreitol or other SH compounds, might be beneficial. Saturating the sample with a protective atmosphere of helium or hydrogen gas, or dropping a small pellet of dry ice in the sample, will usually inhibit free radical formation.

Since the greatest concentration of energy is immediately below the probe, it is imperative that the sample be kept as close to the tip as possible. Liquids are easily processed because the free moving cells circulate repeatedly below the probe. Solid materials, however, have a tendency to be repelled by the ultrasonics, and should be processed in a vessel large enough to accommodate the probe, yet small enough to restrict sample movement. For small samples, conical shaped test tubes are recommended. Although plastic tubes work well, glass and stainless steel tubes are somewhat more effective than plastic ones because they do not absorb the vibrations.

Allowing the probe to come in contact with the vessel will decrease the power output, and cause tiny glass particles to migrate into the liquid. Although these glass particles will not adversely affect the chemical composition of the sample, they will form a thin grey layer on centrifuging. If the probe has to come in contact with a solid sample, use a standard 20 mm (³/₄" diameter stainless steel centrifuge tube cut to 70mm (3") length. Do not use a glass tube. Microtips must never be allowed to come in contact with anything but the liquid, because the stress resulting at the point of contact with the vessel will cause the microtip to fracture. Although larger probes will not fracture if they come in contact with the treatment vessel, they may cause the vessels to fracture.

Cell disruption can be enhanced by increasing hydrostatic pressure (typically 15-60 psi) and viscosity. For microorganisms, the addition of glass beads in the 0.05 to 0.5 mm size range promotes cell disruption by focusing the energy released by cavitation, and by physical crushing. Beads are almost a prerequisite when disrupting spores and yeast. A good ratio is one volume of beads to two volumes of liquid. Glass bead are available from Catatphote, Inc. P.O. Box 2369, Jackson, Mississippi 39225-2369 USA, phone (800) 221-2574 or (601) 939-4612, FAX (601) 932-5339, Jayco Inc., 675 Rahway Ave., Union, NJ 07083 USA, phone (908) 688-3600, FAX (908) 688-6060, or Sigmund Lindner GmbH. P.O. Box 29. D-95483 Warmensteinach, Germany. Phone (49) 0 92 77 9 94 10, FAX (49) 0 92 77 9 94 99.

When processing difficult cells, pretreatment with an enzyme such as lysozyme or byaluronidase might be beneficial. Glusulase has been used successfully with yeast, lysostaphin with staphylococcus, collagenase with skin and cartilage, and trypsin hyaluronidase with liver and kidney.

If enzymes can not be used, the following procedures should be considered: Freezing the sample at -70°C overnight, then thawing in ice water immediately prior to sonication.

Whenever possible, the tissues should be diced very small to permit movement within the liquid. Tough tissue such as skin and muscle should be macerated first in a blender or the like for about 10 seconds, and confined to a small vessel during ultrasonic treatment. Freezing followed by powdering could also be resorted to if this procedure is not detrimental to the experiment. If sub-cellular particles are desired intact, the amplitude control should be set low and the processing time increased.

Insert the probe deep enough below the surface of the sample to inhibit aerosoling or foaming. Foaming substantially reduces cavitation and can cause protein denaturation. Processing at a lower power setting without foam is much more effective than processing at a higher power setting with foam. Decreasing the power, increasing

SECTION II - OPERATION

PRINCIPLES OF ULTRASONIC DISRUPTION

The ultrasonic power supply converts 50/60 Hz line voltage to high frequency electrical energy at 20 kHz or 40 kHz (20,000 or 40,000 cycles per second), depending on the model. This high frequency electrical energy is transmitted to the piezoelectric transducer within the converter, where it is changed to mechanical vibrations. The vibrations from the converter are intensified by the probe, creating pressure waves in the liquid. This action forms millions of microscopic bubbles (cavities) which expand during the negative pressure excursion, and implode violently during the positive excursion. It is this phenomenon, referred to as cavitation, which produces the powerful shearing action at the probe tip, and causes the molecules in the liquid to become intensely agitated.

NOTE

The Ultrasonic Processor is available with five probes - a 2 mm ($5/64$ ") microtip, a 3 mm ($1/8$ ") microtip, a 6 mm ($1/4$ ") microtip, a 9.5 mm ($3/8$ ") microtip, and a 13 mm ($1/2$ ") probe.

The 2 mm microtip is optional, and at 20 kHz can process between 100 microliters and 5 milliliters.

The 3 mm microtip is standard with the 70 and 100 watt model, and at 20 kHz can process between 200 microliters and 10 milliliters.

The 6 mm microtip is standard with the 130 and 150 watt model, and at 20 kHz can process between 10 and 25 milliliters.

The 9.5 mm microtip is optional, and at 20 kHz can process between 25 and 50 milliliters.

The 13 mm probe is optional, available with or without replaceable tip, and at 20 kHz can process between 50 and 150 milliliters.

FUNCTIONS OF CONTROLS, INDICATORS, AND CONNECTORS

	FRONT PANEL
AMPLITUDE control	When depressed, switches the main power on or off. When rotated, controls the amplitude of vibrations at the probe tip.
Pulsing button*	Located on the converter. When depressed activates the ultrasonics.
PULSE/CONTINUOUS switch*	Select the application of ultrasonics on the pulse or continuous mode.
TIMER**	Sets the duration of ultrasonic application from 1 minute to 10 minutes. When depressed, activates the ultrasonics for the length of the timing sequence selected.
PULSER**	Activates the ultrasonics on a pulsed mode. Pulse duration can be varied from 1 second OFF/1 second ON, to 1 second OFF/10 seconds ON. OFF cycle is always 1 second. ON cycle is variable from 1 second to 10 seconds. In the OFF position the ultrasonics is continuous. Intermittent operation inhibits heat build-up in the liquid and provides more efficient processing by allowing the material to settle back under the probe tip after each burst.
WATTMETER	Indicates in watts the amount of ultrasonic power delivered to the probe.

*For 70 and 100 watt model only.

**For 130 and 150 watt model only.

OPERATING SUGGESTIONS AND TECHNIQUES

DISRUPTING CELLS

Single-cell organisms (microorganisms) consist of a semipermeable, tough, rigid, outer cell wall surrounding the protoplasmic (cytoplasmic) membrane and cytoplasm. The cytoplasm is made up of nucleic acids, proteins, carbohydrates, lipids, enzymes, inorganic ions, vitamins, pigments, inclusion bodies, and about 80% water. In order to isolate and extract any of these substances from inside the cell, it is necessary to break the cell wall and protoplasmic membrane. In some cases, the cells may excrete the desired substance, but, in most cases, the cell wall must be ultrasonically disrupted to release these substances.

Micro-organisms differ greatly in their sensitivity to ultrasonic disintegration. For example, the most readily disintegrated are the rod-like forms (bacilli), while the spherical organisms (cocci) are much more resistant. The group *Mycobacteria*, to which the tuberculosis organism belongs is particularly difficult to disrupt. Generally, animal cells are more easily disintegrated than plant cells, and red blood cells are more readily disintegrated than muscle cells because they lack a protective cell wall.

With ultrasonic processing, the molecular motion in the sample will usually cause temperature elevation — especially with small volumes. Since high temperature reduces cavitation, the sample temperature should be kept as low as possible — preferably just above its freezing point. This can be accomplished by immersing the sample vessel in an ice-salt-water bath. Temperature elevation can also be minimized by using the pulser or subjecting the sample to several short bursts.

CAUTION

- Do not operate the power supply unless it is connected to the converter.
- High voltage is present in the power supply — Do not operate with the cover removed.
- Never allow a microtip to vibrate in air for more than 10 seconds.
- Do not allow the vibrating probe to come in contact with anything but the sample.

1. Immerse the probe into the sample.
2. For continuous operation with the 70 and 100 watt model, set the PULSE/CONTINUOUS switch to CONTINUOUS, then set the AMPLITUDE control to 40.
3. For pulsed operation with the 70 and 100 watt model: 1) Set the PULSE/CONTINUOUS switch to PULSE. 2) Set the AMPLITUDE control to 40. 3) Depress the thumb activated pulsing button on the converter.
4. For continuous operation with the 130 and 150 watt model set the AMPLITUDE control to 40.
5. For pulsed operation with the 130 and 150 watt model, use the optional footswitch or the PULSER.
6. For timed operation with the 130 and 150 watt model: 1) Set the TIMER as required. 2) Set the AMPLITUDE control to 40. 3) Depress the TIMER knob.
7. If necessary, rotate the AMPLITUDE control to increase or decrease the intensity as required.

IMPORTANT

Proper care of the probe is essential for dependable operation. The intense cavitation will, after a prolonged period of time, cause the tip to erode, and the power output to decrease without showing up on the wattmeter. The smoother and shinier the tip, the more power will be transmitted into the sample. Any erosion of the probe tip will increase the rate of future erosion. For that reason it is recommended that after every 5 or 6 hours of use the tip be examined, and if necessary, polished with emery cloth or an abrasive wheel. Since the probe is tuned to vibrate at a specific frequency, it is most important that only the contaminated surface be removed. This procedure can be repeated as long as the wattmeter reads less than 20 watts with the probe out of the sample, and the AMPLITUDE control set at 100. Then the probe or replaceable tip should be changed.

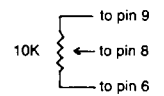
FUNCTIONS OF CONTROLS, INDICATORS, AND CONNECTORS (cont.)

REAR PANEL	
9 pin D-sub connector	Connects to external actuation device, and enables remote power and frequency monitoring.
Footswitch jack*	Connects to the footswitch cable.
4 pin connector	Connects to the converter with thumb activated pulsing button.**
BNC connector	Connects to the converter without thumb activated pulsing button.
Power module	Connects to the electrical line cord and houses fuse(s) for AC line protection.

*The 70 and 100 watt model can be used either with a footswitch or a converter with a pulsing button. Not with both. A standard converter must be used with a footswitch.
**For 70 and 100 watt model only

9-PIN D-SUB CONNECTOR

Pin No.	Description
1	Not connected
2	Not connected
3	Not connected
4	Enables connection to a frequency counter.
5	Enables connection to an external power monitor (10 mv = 1 watt).
6	Ground
7	Energizes the ultrasonics when connected to ground.
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 the positive to pin 8 and the negative to pin 6.

PREPARATION FOR USE

CAUTION

If the Ultrasonic Processor has been left in a very cold or hot environment for a prolonged period of time, do not operate until it has reached room temperature.

1. Ensure that the AMPLITUDE control is set to OFF.
2. Plug the electrical cord into an electrical outlet.
3. If the probe is not already assembled, observe step 4.
4. Hand assemble the probe to the converter, and using wrenches provided, tighten securely.

CAUTION

Never assemble or disassemble a probe by holding the converter in a vise.

5. Mount the converter/probe assembly in a lab stand. Secure the clamp to the 1 1/4" (32 mm) diameter converter housing only.
6. Connect the converter cable to the power supply.
7. If the optional footswitch is used, insert the footswitch plug into the jack on the rear panel. Make sure that the plug is inserted forcefully all the way in.

USING THE ULTRASONIC PROCESSOR



The Speed Control on an automobile, can, to a certain extent, be compared to an Ultrasonic Processor. This device is designed to maintain the vehicle rate of travel constant. As the terrain changes, so do the power requirements. The Speed Control senses these requirements, and automatically adjusts the amount of power delivered by the engine, in order to compensate for these ever changing conditions. The steeper the incline, the greater the resistance to the movement of the vehicle, and the greater the amount of power that will be delivered by the engine, to overcome that resistance.

The Ultrasonic Processor is designed to deliver constant amplitude. As the resistance to the movement of the probe increases, so do the power requirements. The power supply senses these requirements, and automatically increases the amount of power delivered, in order to maintain the excursion at the probe tip constant. Under identical loading conditions, the wattage delivered by two power supplies with different power ratings will be identical (provided both have sufficient power capability).

The AMPLITUDE control allows the ultrasonic vibrations at the probe tip to be set to any desired level. Although the degree of cavitation required to process the sample can readily be determined by visual observation, the amount of power required cannot be predetermined. A sensing network continuously monitors the output requirements, and automatically adjust the power to maintain the amplitude at the preselected 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 fully clockwise will not cause the maximum power to be delivered to the sample. The maximum power that the Ultrasonic Processor is capable of delivering, will only be delivered when the resistance to the movement of the probe is high enough to draw maximum wattage.

This phenomenon can be demonstrated as follows: Depress the probe down against a piece of wood. The greater the down pressure, and consequent greater resistance to the movement of the probe, the greater the amount of power that will be delivered by the power supply.