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USER'S GUIDE

AUTOTUNE SERIES
HIGH INTENSITY ULTRASONIC PROCESSOR WITH TEMPERATURE CONTROLLER

250 Watt Model • 500 Watt Model
600 Watt Model • 750 Watt Model

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The Ultrasonic Processor supplied with this instruction manual is constructed of the finest material and the workmanship meets the highest 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.

Rev. 01 6/28/02

WARRANTY

Your Ultrasonic Processor is warranted and backed by the manufacturer for a period of **three 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, the manufacture will, at its option, as the exclusive remedy, either repair or replace without charge for material and labor, the part(s) which 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 date of shipment. A defective probe will be replaced once 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.

This warranty is in lieu of any other warranties, either express, implied, or statutory. The manufacturer neither assumes nor authorizes any person to assume for it any other obligations or liability in connection with the sale of its products. The manufacturer hereby disclaims any warranty of either 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 the manufacturer be liable to the purchaser or any other person for any incidental or consequential damages or loss of goodwill, production, or profit resulting from any malfunction or failure of its product.

This warranty does not apply to equipment that has been subject to unauthorized repair, misuse, abuse, negligence or accident. Equipment which, shows evidence of having been used in violation of operating instructions, or which has had the serial number altered or removed, will be ineligible for service under 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. The manufacturer assumes no responsibility for probes fabricated by another party or for consequential damages resulting from their usage.

The aforementioned provisions do not extend the original warranty period of any product that has either been repaired or replaced by the manufacturer.

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IMPORTANT SAFEGUARDS

READ BEFORE INSTALLING OR USING THE EQUIPMENT

Your Ultrasonic Processor has been designed with safety in mind. 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 operating the equipment, and retain this instruction manual for future reference. If the ultrasonic Processor is used in a manner contrary to that specified in this instruction manual, the protection features designed into the unit may be impaired.

- When mounting the probe, always clamp the converter housing. Never clamp the probe.
- Make sure the Ultrasonic Processor is properly grounded via a 3-prong outlet.
- High voltage is present in the power supply. Do not remove the cover. Refer all servicing to qualified service personnel.
- To avoid electric shock, disconnect the electrical power cord before removing the cover prior to servicing.
- Never operate the power supply unless it is connected to the converter.
- Never secure anything to the probe, except at the nodal point (point of no activity).
- Never touch a vibrating probe.
- Never allow a microtip or extender to vibrate in air for more than 10 seconds.
- When using a microtip, always keep the amplitude below 40%.
- Never operate a probe with threaded end without a tip, extender or microtip.
- Air-cool the converter when sample temperature exceeds 100° C, and when working at high intensity for more than 30 minutes.
- It is recommended that a sound abating enclosure or ear protection be used when operating the Ultrasonic Processor.

WARNING or CAUTION

Where you see this alert symbol and WARNING or CAUTION heading, strictly follow the warning instructions to avoid personal injury or equipment failure.

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CAUTION

LOW SURFACE TENSION LIQUIDS – ORGANIC SOLVENTS

The probes (solid or with a replaceable tip) are tuned elements that resonate at a specific frequency. If the replaceable tip is removed or isolated from the rest of the probe, the element will no longer resonate at that frequency, and the power supply will fail.
Unlike aqueous (water based) solutions which rarely cause problems, solvents and low surface tension liquids are problematic. These liquids penetrate the probe/replaceable tip interface, and force the particulates into the threaded section isolating the tip from the probe.
When processing low surface tension liquids ALWAYS use a solid probe

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SECTION 1 – 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. 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.

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ELECTRICAL REQUIREMENTS

The Ultrasonic Processor requires a fused, single phrase 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.

WARNING

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

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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.

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SECTION II – OPERATION

PRINCIPLES OF ULTRASONIC DISRUPSION

The ultrasonic power supply converts 50/60 Hz line voltage to high frequency electrical energy. 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. This phenomenon, referred to as cavitation, creates millions of shock waves in the liquid, as well as elevated pressures and temperatures at the implosion sites. Although the cavitational collapse last but a few microseconds, and the amount of energy released by each individual bubble is minute, the cumulative effect causes extremely high levels of energy to be released into the liquid. The larger the probe tip, the larger the volume that can be processed but at a lesser intensity. For information regarding the processing capability of each probe, consult the tables below.

TAPERED MICROTIPS				STEPPED MICROTIP
TIP DIAMETER	1/8" (3mm)	3/16" (5mm)	1/4" (6.5mm)	1/8" (3mm)
INTENSITY	ultra high	very high	high	very high
VOLUME (batch)	1-10ml	3-20ml	5-50ml	250ul-10ml

STANDARD PROBES			
TIP DIAMETER	1/2" (13mm)	3/4" (19mm)	1" (25mm)
INTENSITY	high	medium	low
VOLUME (batch)	10-250ml	25-500ml	500-1000ml

HIGH GAIN PROBES		
TIP DIAMETER	3/4" (19mm)	1" (25mm)
INTENSITY	high	medium
VOLUME (batch)	25-500ml	500-1000ml

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FUNCTIONS OF KEYS, CONTROLS, INDICATIONS, AND CONNECTORS

FRONT PANEL	
LCD screen	Displays prompts and the following control parameters: • Amplitude selected • Output power delivered to the probe in watts, and as percentage of the total power • Selected duration of processing • Actual processing time • Elapsed time • Set and read temperature • Pulse duration • Accumulated amount of energy in Joules delivered to the probe.
0 – 9 key	Input digits.
CLEAR key	Clears preceding entry.
ENTER REVIEW key	Enters data into the program, and selects various parameters, for display on the LCD screen.
TIMER key	Used with the numeric keys to set the duration of ultrasonic application – from 1 second to 9 hours, 59 minutes, 59 seconds.
TEMP key	Used with the numeric keys to set the high temperature limit – from 1°C to 99°C. Red indicator lights when the temperature limit has been reached.
PULSER key	Used with the numeric keys to set the pulse mode. The ON cycle and OFF cycle can be set independently from .1 second to 9.9 seconds. Red indicator lights when pulser is in the OFF portion of the cycle.
START/STOP Key	Starts or stops the ultrasonics. In the STOP mode the red indicator goes off.
PAUSE key	Suspends operation. Red indicator lights when the processing cycle is interrupted.
RECALL Key	Used with numeric keys to recall any of 10 stored programs. Lit red indicator signals that a program identification number must be entered.
SAVE key	Used with the numeric keys to assign a number to a program and store that program in memory. Up to 10 programs (0-9) can be stored. Lit red indicator signals that a program identification number must be entered.
ON/OFF power switch (located below the control panel)	Switches the main power on or off.
AMPLITUDE control (located below the control panel)	Controls the amplitude of vibration at the probe tip. CAUTION *When using a microtip, never allow the amplitude to exceed 40%*

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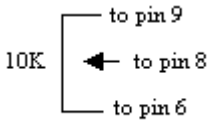
FUNCTIONS OF KEYS, CONTROLS, INDICATIONS, AND CONNECTORS (cont.)

REAR PANEL	
9 pin D-sub connector	Connects to external actuation device, and enable power and frequency monitoring.
Footswitch jack	Connects to the footswitch cable.
Coax connector	Connects to the converter.
Power module	Connects to the electrical line cord and encases the fuse(s).

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 (5 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. *See below*



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NOTE

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

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PREPARATION FOR USE

CAUTION

Do not operate an Ultrasonic Processor that has been in a very cold or hot environment for a prolonged period of time. Wait until it has reached room temperature

1. Ensure that the AMPLITUDE dial is set fully counter-clockwise.
2. Plug the electrical line cord into the electrical outlet.
3. If the optional footswitch is used, insert the plug into the jack located on the rear panel. Make sure that the plug is inserted forcefully all the way in.
4. If the converter / probe assembly is not already assembled, check for cleanliness the mating surface of the converter and probe or stepped microtip (consisting of coupler and stepped tip), and using the wrenches provided, screw them securely to the converter.
5. To attach a tapered microtip or extender to a probe, remove the replaceable tip from the ½" (13mm) probe, and using the wrenches provided, screw them securely to the probe.

CAUTION

Never place a washer between the probe and the converter.
Never apply grease to the mating surfaces or threads of the converter, probe, replaceable tip or microtip.

6. Mount the converter / probe assembly in a laboratory stand. Secure the clamp to the 2 ½" (63mm) diameter converter housing only. Do not secure the clamp to the probe.
7. Connect the converter cable to the power supply.

NOTE

Should it become necessary to remove a probe, use the wrenches supplied. If the probe has been attached to the converter for a long period of time it might be necessary to use a vise. Be sure the vise has soft jaws or other means to prevent scratching. Secure the wide diameter portion of the probe in the jaws of the vise. Never grip the converter in the vise. Using a wrench, twist the converter off the probe. A tap of a hammer may be applied to the end of the wrench. Never attempt to remove the probe by twisting the converter housing, as this may damage the electrical connections within the housing.

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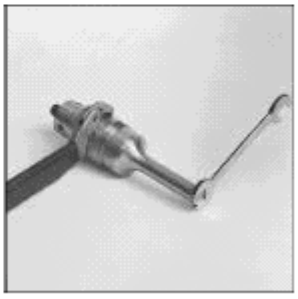
REMOVAL

TIGHTENING

TIP REMOVAL



TIP TIGHTENING

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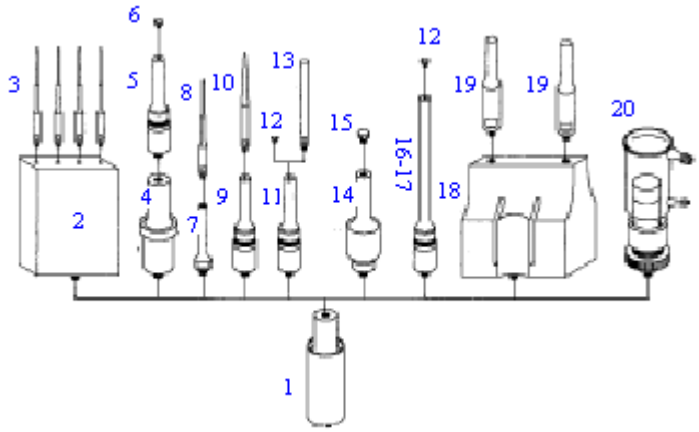

No	DESCRIPTION	Order Number
1	Converter Model CV33	CV00033
2	Four element coupler	630-0558
3	Stepped top(s) $^{1}/_{8}$" (3mm)	630-0535
4	Booster	BHNVCGD
5	Probe ½" (13mm) solid	630-0219
	Probe ½" (13mm) with threaded end and replaceable tip	630-0220
	Probe ¾" (19mm) solid	630-0208
	Probe ¾" (19mm) with threaded end and replaceable tip	630-0207
	Probe 1" (25mm) solid	630-0209
	Probe 1" (25mm) with threaded end and replaceable tip	630-0210
6	Replaceable tip ½" (13mm)	630-0406
	Replaceable tip ¾" (19mm)	630-0407
	Replaceable tip 1" (25mm)	630-0408
7	Coupler	630-0421
8	Stepped tip 1/8" (3mm)	630-0422
9	Probe ½" (13mm) with threaded end and replaceable tip	630-0220
10	Tapered microtip $^{1}/_{8}$" (3mm)	630-0418
	Tapered microtip $^{3}/_{16}$" (5mm)	630-0419
	Tapered microtip ¼" (6mm)	630-0420
11	Probe – solid or with threaded end and replaceable tip – same as 5	
12	Replaceable tip – same as 6	
13	Extender ½" (13mm)	630-0410
	Extender ¾" (19mm)	630-0409
	Extender 1" (25mm)	630-0444
	Full wave extender ¾" (19mm) – 10" (254mm) long	630-0518
	Full wave extender 1" (25mm) – 10" (254mm) long	630-0519
14	High gain probe ¾" (19mm) – solid	630-0306
	High gain probe ¾" (19mm) with threaded and replaceable tip	630-0305
	High gain probe 1" (25mm) – solid	630-0310
	High gain probe 1" (25mm) with threaded and replaceable tip	630-0311
15	Replaceable tip ¾" (19mm) or 1" (25mm) – same as 6	
16	Full wave probe ½" (13mm) solid – 10" (254mm) long	630-0217
17	Full wave probe ½" (13mm) – 10" (254mm) long with threaded and replaceable tip	630-0218
18	Aluminum coupler	630-0562
19	¾" (19mm) solid probe	630-0208
20	Cup horn 1 ½" (38mm)	630-0503
	Cup horn 2 ½" (64mm)	630-0431
	Cup horn 3" (76mm)	630-0496

CAUTION: Do not use tapered microtip with coupler. Do not use stepped tip without a coupler. Do not use probes with threaded end and replaceable tip, when working with low surface tension liquids.

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USING THE ULTRASONIC PROCESSOR

The speed control on an automobile, can, to a certain extent, be compared to an Ultrasonic Processor. The speed control is designed to maintain the vehicles 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 greater the terrain rate of incline and greater the resistance to the movement of the vehicle, 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, additional power will be delivered by the power supply to ensure that the excursion at the probe tip remains constant. Using a more powerful power supply will not deliver more power into the liquid. Rather, it is the resistance to the movement of the probe that determines how much power will be delivered into the sample.

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 adjusts 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.

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CAUTION

- Never allow liquid to spill into the converter. Do not use the cup horn without a splash shield
- Do not allow a microtip or extender to vibrate in air for more than 10 seconds. When working with a microtip never allow the AMPLITUDE control to be set above the microtip limit 40%. Ignoring these instructions will cause the microtip to fracture.
- Do not allow the vibrating microtip to contact anything but the sample.
- When working with low surface tension liquids, do not use a probe with a replaceable tip.
- Never energize a threaded probe without the replaceable tip, extender, or microtip attached.

NOTE

Refer to Section IV, for general operating suggestions and ultrasonic processing techniques.

1. Set ON/OFF power switch to ON. The switch will illuminate and the screen will display the power rating of the Ultrasonic Processor, cautionary notices, and the following control parameters.

TIME __:__:__	TEMP __ __°C
PULSE__:__:__	AMPL __ __%

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AMPLITUDE: The amplitude is the only parameter that must be set in order for the Ultrasonic Processor to be operational. The other control parameters – Time and Pulse, do not have to be set for continuous operation. AMPL. displays the percentage of maximum of amplitude e.g. 40%, set by the AMPLITUDE control. Rotate the AMPLITUDE control for a 40% reading on the LCD screen – Ampl 40%.

The screen will display:

TIME __:__:__	TEMP __ __°C
PULSE__:__:__	AMPL 40 %

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The Ultrasonic Processor is now ready for continuous operation. To energize ultrasonics, press the START key or the footswitch. To de-energize ultrasonics, press the STOP key or release the footswitch. If the Time, Temperature, Pulse, Save, or Recall functions must be used, refer to the appropriate paragraph(s) below.

NOTE

Any combination of functions can be selected in any order. To clear an erroneous entry press the CLEAR key.

NOTE

If the START key is pressed and the time limit has not been set, processing will remain uninterrupted until the STOP key is depressed.

If the START key is pressed and the time limit has been set, processing will remain uninterrupted until the set time limit expires, or the STOP key is pressed – whichever occurs first.

If a footswitch is use, and the time limit has not been set, processing will remain uninterrupted as long as the footswitch is depressed.

If a footswitch is used, and the time limit has been set processing will remain uninterrupted until the time limit expires or the footswitch is released – whichever occurs first.

The START 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.

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NOTE

The probe is tuned to vibrate at a specific frequency. If the resonant frequency of the probe has changed, due to cavitation erosion or fracturing, a minimum reading will not be obtained. If an overload condition exits, or if minimum reading cannot be obtained (less than 20%) with the probe out of the sample, check the instrument without the prove to determine which component might be defective. If minimum reading is obtained using the converter without the probe, the probe is defective and should be changed.
A loose probe will usually generate a loud piercing sound.
Refer to Section III if an overload condition exists.

Immerse the probe approximately 2 inches (5cm) into the sample. If a microtip is used, immerse the microtip approximately ½" (1cm) into the sample. If the probe is immersed to an insufficient depth, air will be injected into the sample, causing the sample to foam. Since the amplitude required is application dependent and subject to the volume and composition of the sample, it is recommended that the amplitude be increased or decreased as required as the sample is being processed.

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

The screen will display:

Time Setting Hrs:__ Min:_ _ Sec:_ _

Using the numeric keys, set the processing time as required:

e.g

Time Setting Hrs: 5 Min: 30 Sec: 25

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

TIME 5:30:25	TEMP __ __°C
PULSE__:__:__	AMPL 40 %

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PULSER: By inhibiting heat build-up in the sample, the pulse function enables safe treatment of temperature sensitive samples at high intensity. In addition, pulsing enhances processing by allowing the material to settle back under the probe after each burst. The ON and OFF pulse duration can be set independently from .1 second to 9.9 seconds. During the OFF portion of the cycle, the red indicator on the PULSER key will illuminate. If the OFF portion of the cycle exceeds two seconds, a cautionary message - CAUTION – PROBE ON STANDBY – will warn the operator against touching the ultrasonic probe. To set the pulser, press PULSER key.

The screen will display:

Pulse on __.__sec Pulse off __.__sec

Using the numeric keys, set the ON portion of the cycle, and press the ENTER/REVIEW key.

The screen will display:

e.g.

Pulse on 2.5 sec Pulse off __.__sec

Using the numeric keys set the OFF portion of the cycle.

The screen will display:

e.g.

Pulse on 2.5 sec Pulse off 1.0 sec

Press the ENTER/REVIEW key.

The screen will display:

TIME 5:30:25	TEMP __ __°C
PULSE 2.5 : 1.0	AMPL 40 %

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TEMPERATURE: The temperature function prevents overheating of the sample by continuously monitoring the sample temperature, and terminating the ultrasonics when the temperature reaches a predetermined setpoint. The ultrasonics is automatically reinstated when the temperature drops below the setpoint. If the temperature of the sample must be monitored and /or controlled, insert the optional Temperature Probe forcefully into the small jack on the rear panel, immerse the Temperature Probe in the sample and Press the TEMP key.

The screen will display:

e.g.

Probe Temperature 27° C Temperature Setpoint __° C

Using the numeric keys set the high temperature limit (setpoint).

The screen will display:

e.g.

Probe Temperature 27° C Temperature Setpoint 35° C

Press the ENTER/REVIEW key.

The screen will display:

TIME 5:30:25	TEMP 35° C
PULSE 2.5 : 1.0	AMPL 40 %

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

a) Selected amplitude:
   e.g. Amplitude Control 40%
b) Set and read temperature:
   e.g. Temp Set 35°C Probe 27°C
c) Set processing time and elapsed processing time:
   e.g. Set 5:30:25 Time 0:57:03
d) Selected pulsing cycle, and actual pulsing cycle:
   e.g. Pulse 2.5 1.0/1.5 .5
e) Amount of power in watts, and accumulated amount of energy in JOULES delivered to the probe:
   e.g. 20 watts 0000000 Joules
f) Elapsed time since processing was initiated:
   e.g. Elapsed time 1:27:33

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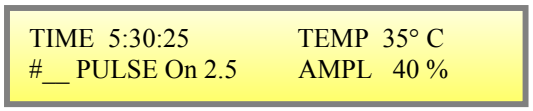
NOTE

The amount of energy displayed will be only for one cycle. Initiating a new cycle will reset the display to zero.

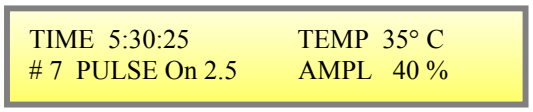
SAVE: The save function retains in memory up to 10 control parameters under a storage identification (ID) number. To store the parameters under an ID number, (0-9) press the SAVE key. The indicator light on the SAVE key will illuminate and the screen will display.

e.g.



Using the numeric keys, enter the ID number.

e.g.



Press the ENTER/REVIEW key to store the control parameters under the assigned ID number. The indicator light on the SAVE key will go out, and the screen will display the parameters stored under that ID number:

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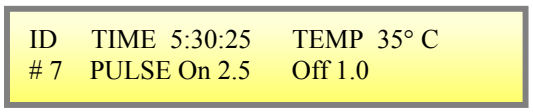
RECALL: The recall function can retrieve from memory; any of the 10 stored control parameters for verification or usage. To retrieve any parameters press the RECALL key. The indicator light on the RECALL key will illuminate, and the screen will display:

e.g.

Using the ID number and the numeric keys, select the desired parameters, which must be retrieved. The screen will display:

e.g.



To retrieve from memory the parameters stored under that ID number, press the ENTER/REVIEW key. The screen will display:

NOTE

To review all the information that has been stored, press keys 0 to 9 one at a time.

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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, when the AMPLITUDE control is set at 100. If the wattmeter reads over 20 watts the probe or replaceable tip should be replaced with a new one.

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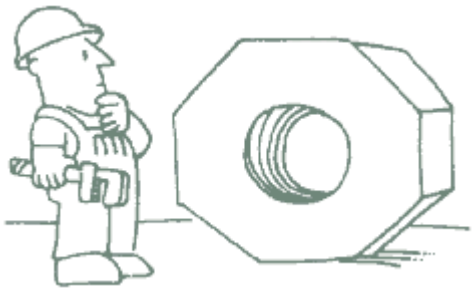
SECTION III – SERVICE INFORMATION



Your Ultrasonic Processor 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 due to an overload condition or that it will stop working all together. The most probable causes for malfunction are listed below and should be investigated.

- The unit was plugged into an electrical outlet that provides a different voltage from that required. See *Electrical Requirements*.
- The probe and/or microtip is not secured properly.
- If the probe has a replaceable tip, the tip is not secured properly, or the probe has been used with low surface tension liquids.
- A fuse(s) has failed. If a fuse(s) has failed, proceed as follows:
  1. Ensure that the power switch is set to OFF.
  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. Set the AMPLITUDE control to 50 and the power switch to ON. With the probe in air (out of sample), the wattmeter should read below 20 watts. If the reading exceeds 20 watts, set the power switch to OFF, and disconnect the probe from the converter.
  5. Set the power switch back to ON. If the wattmeter reads below 20 watts, the probe has failed or is out of tune due to excessive erosion, and should be replayed, if the wattmeter reads above 20 watts, either the converter or power supply has failed and the complete Ultrasonic Processor should be returned for repair.
  6. If the Ultrasonic Processor stops working due to an overload condition as indicated on the display, investigate and remedy the problem, then set the power switch to OFF then back to ON to reset the instrument.

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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 CERTICATION CAN BE MADE.

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TEMPERATURE PROBE CALIBRATION

Ultrasonic Processors shipped with the optional Temperature Probe have been calibrated as a set. If the Temperature Probe is acquired separately, calibration should be performed in accordance with the procedure outlined below.

NOTE

For optimum accuracy, the Temperature Probe and ultrasonic Processor should be calibrated as a set.

To calibrate the Ultrasonic Processor, proceed as follows:

1. Fill a 500 ml vessel with approximately 50% ice and 50% water. Allow 5 minutes for the water temperature to stabilize.
2. Fill another 500ml vessel with boiling water, and maintain boiling condition with an immersion heater or other heating device.
3. Forcefully insert the Temperature Probe into the small jack on the rear panel.
4. While holding the TEMP key depress, set the ON/OFF power switch to ON.

The switch will illuminate and the screen will display the following message:

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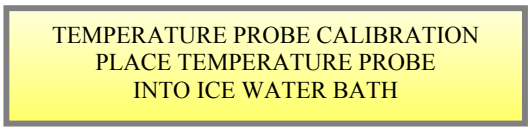
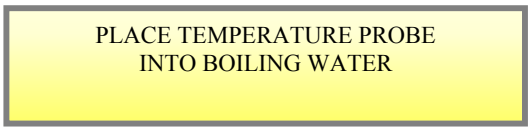


5. Immerse the temperature Probe in the center of the ice water bath for a period of 40 seconds. Do not allow the probe to contact the vessel. When the self-calibration for low temperature is complete, the screen will display the following message:



6. Immerse the Temperature Probe in the center of the boiling water for a period of 40 second. Do not allow the probe to contact the vessel.

When the calibration for high temperature is complete, the screen will display the following message:

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SECTION IV - OPERATING SUGGESTIONS AND TECHNIQUES

DISRUPTING CELLS

Single-cell organisms (micro-organisms) consist of a semipermeable, tough, rigid outer cell wall surrounding the protoplasmic membrane and cytoplasm. The cytoplasm is made up of nucleic acid, protein, 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 cell may excrete the desired substance without assistance, but in most cases, the cells must be lysed in order for these substances to be released. Breaking cell membranes and releasing the contents present significant challenges. The process must be fast and thorough to maximize the protein yield. Because the energy applied must be great enough to break the cell membranes or walls, yet gentle enough to avoid physically or chemically damaging cell content, the Vibra-Cell with its variable intensity capability is ideally suited for this application.

The level of intensity that should be used is application dependent. For example high intensity might be recommended for the break up of cells, but should never be used when the release of intracellular components might be objectionable e.g. Organelle isolation.

Gram negative bacteria typically require 10 to 15 minutes of processing, while staphylococcus requires 20 to 30 minutes.

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 that plant cells, and red blood cells are more readily disintegrated than muscle cells because they lack a protective cell wall.

Ultrasonic processing will typically cause the temperature of the sample to increase especially with small volumes. Since high temperatures inhibit 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.

Increasing hydrostatic pressure (typically 15-60 psi) and viscosity can enhance cell disruption. For micro-organisms, the addition of glass beads in the 0.05 to 0.5mm size range promotes cell disruption. Beads are almost a prerequisite when working with spores and yeast. A good ratio is one volume of beads to two volumes of liquid. Glass beads are available from Cataphote, Inc. P.O. Box 2369, Jackson, Mississippi 39225-

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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. Glycosidase has been used successfully with yeast, lysostaphin with staphylococcus, collagenase with skin and cartilage, and trypsin hyaluronidase with liver and kidney.

If enzymes cannot be used, the following procedures should be considered: Freezing the sample at -70°C overnight, then thawing it in water immediately prior to ultrasonic processing.

Whenever possible, the tissues should be diced very small to permit movement within the liquid. Tough tissues 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. If sub-cellular particles are desired intact, the amplitude should be kept low, and the processing time increased.

Always immerse the probe deep enough below the surface of the sample to inhibit aerosoling or foaming, foaming substantially reduces cavitation. 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 processing time and lowering the temperature of the sample will usually prevent aerosoling and foaming. Do not use any antifoaming agents or surfactants.

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 longer durations, the addition of free radical scavengers such as, carbon dioxide, N_2O, cysteine, reduced glutahione, 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 also inhibit free radical formation.

The problem of oxidation is a serious one particularly where the study of sulpdhydril enzymes is concerned. This may be partially controlled using free radical traps such as cysteine, reduced gluthathione or comparable substances, or by processing in the presence of an inert atmosphere. Whereas it is true that gas is required for effective cellular disruption, it is not necessary that the vapor phase be oxygen or air since any gas except carbon dioxide will work just as well. e.g. Forcing helium or nitrogen through the sample will also reduce aerobic oxidation.

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Since the greatest concentration of energy is beneath 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 ultrasonic, 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 usually work better than plastic ones.

Allowing the probe to contact the vessel will decrease the power output, and cause minute grey glass particles to migrate into the sample. 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 20mm (3/4") diameter stainless steel centrifuge tube cut to 70mm (3") length. Do not use a glass tube. Microtips must never allowed to come in contact with anything but the liquid, because the stress resulting at the point of contact with a hard surface will cause the microtip to fracture. Although larger probes will not fracture if they come in contact with a glass vessel, they may cause the vessel to fracture.

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

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, USA, phone (314) 771-5765, is ideally suited for that purpose.

Probes may be autoclaved, or sterilized 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. Because with ultrasonics the sound waves are propagated through the sample, if the sample is so thick that it will not pour or circulate easily, it is too thick for ultrasonic processing.

Use the Continuous Flow Cell for processing large volumes. This accessory is recommended for the treatment of low viscosity samples, which do not require extended exposure to ultrasonics. When working with temperature sensitive sample, circulate the sample through a coiled tube immersed in a salted ice bath to minimize the temperature elevation that takes place within the cell.

Use the Cup Horn for processing pathogenic, radioactive, and biohazardous materials in complete isolation without probe intrusion. Because plastic tubes have a tendency to absorb vibrations, it is preferable to contain the sample in a stainless steel tubes or glass tubes when working with a cup horn. To expedite processing, add glass beads to the sample. If desired, crushed ice can also be added to the water inside the cup horn, in order

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to optimize cooling. Processing samples in a Cup Horn will usually take 3 to 4 times longer than processing with direct probe intrusion.

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