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USER'S GUIDE

AUTOTUNE SERIES HIGH INTENSITY ULTRASONIC PROCESSOR

MICROPROCESSOR CONTROLLED
100 Watt Model • 130 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.

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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 manufacturer 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.

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.

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 to vibrate in air for more than 10 seconds.
- Never operate a threaded probe without a replaceable tip.
- 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.



CAUTION

LOW SURFACE TENSION LIQUIDS – ORGANIC SOLVENTS

The probes (solid or with a replaceable tip) are tuned elements that resonate at a specific frequency. When using a ½” (13 mm) probe with a replaceable tip, if the replaceable tip is removed or isolated from the rest of the probe, the probe will no longer resonate at that frequency, and the power supply will fail.

Unlike aqueous (water based) solutions, which rarely cause problems, samples containing solvents or 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 samples containing solvents or low surface tension liquids **ALWAYS** use a solid probe

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.

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 unit.

Should it become necessary 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 display should be 220.
7. Change the electrical power cord as required.



WARNING

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



CAUTION

Operating the equipment with voltages different from those specified, or with voltages with high power transients, will cause components within the power supply to fail.

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.

SECTION II – OPERATION

PRINCIPLES OF ULTRASONIC DISRUPTION

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. As the bubbles implode, they cause millions of shock waves and eddies to radiate outwardly from the site of collapse, as well as generate extremes in pressures and temperatures at the implosion sites. Although this phenomenon, known as cavitation, lasts but a few microseconds, and the amount of energy released by each individual bubble is minimal, the cumulative amount of energy generated is extremely high. 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.

NOTE

The Ultrasonic Processor is available with five probes – a 2 mm ($\frac{5}{64}$ ") microtip, a 3 mm ($\frac{1}{8}$ ") microtip, a 6 mm ($\frac{1}{4}$ ") microtip, a 13 mm ($\frac{1}{2}$ ") probe, and a 13 mm ($\frac{1}{2}$ ") probe with replaceable tip.

- The 2 mm microtip is optional, and can process between 150 microliters and 5 milliliters.
- The 3 mm microtip is optional, and can process between 250 microliters and 10 milliliters.
- The 6 mm microtip is standard, and can process between 10 and 25 milliliters.
- The 13 mm probe is optional, available with or without replaceable tip, and can process between 50 and 150 milliliters.

FUNCTIONS OF KEYS, CONTROLS, INDICATORS, AND CONNECTORS

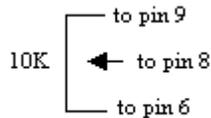
FRONT PANEL	
LCD screen	<p>Displays prompts and the following control parameters:</p> <ul style="list-style-type: none"> ● Amplitude selected ● Amount of output power delivered to the probe in watts, and as a percentage of either 100 watts or 130 watts. ● Selected duration of processing ● Actual processing time ● Elapsed time ● Pulse duration ● Accumulated amount of energy in Joules delivered to the probe.
0 – 9 key	Inputs digits.
CLEAR key	Clears preceding entry.
ENTER REVIEW key	Enters data into the program, and selects various parameters for display on the 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.
PULSE key	Used with the numeric keys to set the pulse mode. The ON cycle and OFF cycle can be set independently from 01 second to 59 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.
 key	Switches the main power on.
O key	Switches the main power off.
AMPL	Controls the amplitude of vibration at the probe tip.
▲ ▼ 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, depress the AMPL key to display Amplitude Setting, then depress the ▲ or ▼ key as required.

FUNCTIONS OF KEYS, CONTROLS, INDICATORS, 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. <i>See below</i>



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.

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. Plug the electrical line cord into the electrical outlet, if the unit is already on; depress the  key.
2. 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.
3. If the converter / probe assembly is not already assembled; screw the probe into the converter and secure with wrenches provided.

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.

4. Mount the converter / probe assembly in a laboratory stand, secure the clamp to the upper section of the converter housing only. **Do not secure the clamp to the probe.**
5. 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. Should it become difficult to disconnect the probe from the converter tap the end of the wrench with a hammer, or for extra torque, elongate the wrench by sliding the end of the wrench into a pipe approximately 40" (1 m) in long. Never attempt to remove the probe by twisting the converter housing, as this may damage the electrical connections within the housing.

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 elevations change, 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 to 100% will not cause the maximum power to be delivered to the sample. The maximum power (100 or 130 watts) 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.

CAUTION

- Do not operate the power supply unless it is connected to the converter.
- Never allow liquid to spill into the converter. Do not use the cup horn without a splash shield
- Do not allow a microtip to vibrate in air for more than 10 seconds.
- Do not allow the vibrating microtip to contact anything but the sample.
- When working with samples containing solvents or low surface tension liquids, do not use a probe with a replaceable tip.
- Never energize a threaded probe without the replaceable tip.

NOTE

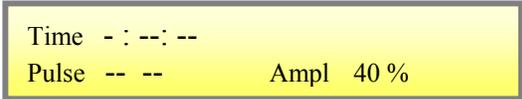
Refer to Section IV, for general operating suggestions and ultrasonic processing techniques.

Press the  key. The screen will display the power rating of the Ultrasonic Processor, and the following control parameters.

Time -- : -- : --	
Pulse -- --	Ampl -- %

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 amplitude selected e.g. 40%. To set the amplitude when the ultrasonics is off, press the **AMPL** key and the numeric keys for a 40% reading on the screen – Ampl 40%, and the **ENTER/REVIEW** key.

The screen will display:



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

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

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 used, 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.

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 exists, or if minimum reading cannot be obtained (less than 20%) with the probe out of the sample, check the instrument without the probe 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 replaced with a new one. A loose probe will usually generate a loud piercing sound. Refer to Section III if an overload condition exists.

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. Since the amplitude required is application dependent and subject to the volume and composition of the sample, it is recommended that the amplitude be selected empirically, by increasing or decreasing the level of intensity 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.

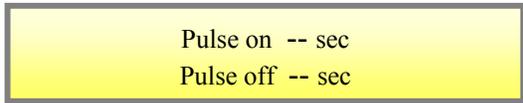
Time Setting
Hrs: 0 Min: 30 Sec: 00

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

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

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 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 ultrasonic probe. To set the pulser, press **PULSE** key.

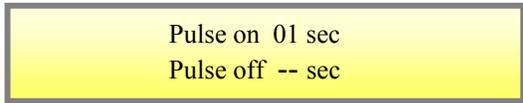
The screen will display:



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

The screen will display:

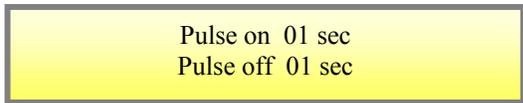
e.g.



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

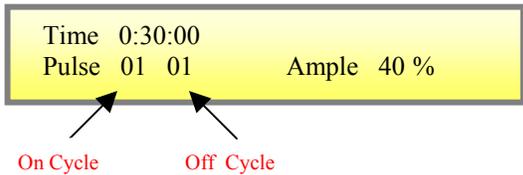
The screen will display:

e.g.



Press the **ENTER/REVIEW** key.

The screen will display:



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 40%
- b) Selected processing time and elapsed processing time:
e.g. Set 0:30:00 Time 0:22:10
- c) Selected pulsing cycle, and actual pulsing cycle:
e.g. Pulse 01 01 / 01 00
- d) Amount of power in watts, and accumulated amount of energy in JOULES delivered to the probe:
e.g. 20 watts 0000000 Joules
- e) Elapsed time since processing was initiated:
e.g. Elapsed time 0:44:20

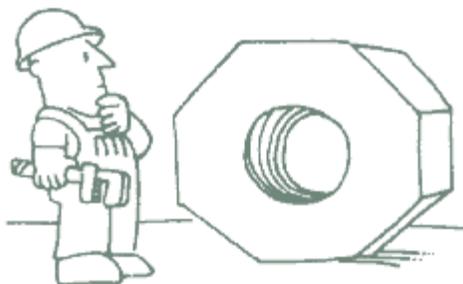
NOTE

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

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 use the tip be examined, and if necessary, polished very carefully 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 10 watts with the probe out of the sample, when the AMPLITUDE is set at 100. If the wattmeter reads over 10 watts, the probe or replaceable tip should be replaced with a new one.

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 samples containing solvents or low surface tension liquids.
- A fuse(s) has failed. If a fuse(s) has failed, proceed as follows:
 1. Turn the unit off by depressing the **O** key, and disconnect the line 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 **|** key and 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, and disconnect the probe from the converter.
 5. Press the **|** key. 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 the complete Ultrasonic Processor should be returned for repair.

OVERLOAD CONDITION

If the Ultrasonic Processor stops working, and an OVERLOAD indication is displayed on the screen, check for possible causes as outlined in the previous section, then press the  key to switch the unit off, and the  key to switch the unit back on.

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 3-year 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.

SECTION IV - OPERATING SUGGESTIONS AND TECHNIQUES

DISRUPTING CELLS

The disruption of cells is an important stage in the isolation and preparation of intracellular products. From research levels through to production, many areas of biotechnology, particularly recombinant technology, necessitate the use of ultrasonics for cell disruption. Although some biological products are secreted from the cell or released during autolysis, many others require sonication to release intracellular material. Cell disruption focuses on obtaining the desired product from within the cell, and it is the cell wall that must be disrupted to allow cell contents extraction.

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 and sonicated 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 contents, 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.

The ability to control the amplitude at the probe tip is a prerequisite for process optimization. And because each application requires its own set of processing parameters, due to variation in volume and composition, the optimum amplitude can only be determined empirically. When processing a new sample, it is recommended that the amplitude be set first at 50% (30% with a microtip) and then increased or decreased as required.

Yeast, gram-positive bacteria, and to a lesser extent, gram-negative bacteria have considerably harder cell walls in comparison to animal cells, and require relatively high power for cell disruption.

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

Microorganisms 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.

Cellular disruption is the first step in RNA isolation and one of the most critical steps affecting yield and quality of the isolated RNA. Typically, cell disruption needs to be fast and thorough. Slow disruption, for example placing cells or tissue in guanidinium isothiocyanate (GITC) lysis solution for a long time prior to sonication, may result in RNA degradation by endogenous RNases released internally. This is especially a concern when working with tissues high in endogenous RNase such as spleen and pancreas.

Disrupting frozen tissue is more time consuming and cumbersome than processing fresh tissue, but freezing samples is sometimes necessary. Samples are usually frozen when, 1) they are collected over a period of time and thus, cannot be processed simultaneously; 2) there are many samples, 3) samples are collected in the field, or 4) mechanical processing of fresh samples is insufficient for thorough disruption. A mortar and pestle or bag and hammer are typically used when the starting material is frozen. RNA will remain intact in tissues for a day at 37°C, a week at 25°C a month at 4°C and indefinitely at subzero temperatures.

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 microorganisms, the addition of glass beads in the 0.5 to 1mm 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 39225-2369 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 such as yeast, pretreatment with an enzyme is beneficial. Lysozyme, hyaluronidase, glycosidase, glucalase, lyticase, zymolase and lysostaphin digestion are among the enzymatic methods frequently used with yeast and Lysozyme with bacteria. Enzymatic treatment is usually followed by sonication in a GITC lysis buffer. Collagenase may be used with collagen, lysostaphin with staphylococcus, 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.

Most animal tissues can be processed fresh (unfrozen). It is important to keep fresh tissue cold and to process it quickly (within 30 minutes) after dissection. When working with fresh tissue, the cells must be sonicated immediately at the time the GITC lysis solution is added. This can be done by dispensing the lysing solution in the tube, adding the tissue and immediately sonicating. Samples should never be left sitting in lysis solution, undisturbed. Large samples of hard tissues should be first treated in a blender or a mechanical homogenizer.

Animal tissues that have been frozen after collection should be disrupted by grinding in liquid nitrogen with a mortar and pestle. During this process, it is important that the equipment and tissue remain at cryogenic temperatures. The tissue should be dry and powdery after grinding. Grinding should be followed by thorough sonication in a GITC lysis buffer. Processing frozen tissue in this way is cumbersome and time consuming, but effective.

Cultured cells are normally easy to disrupt. Cells grown in suspension are collected by centrifugation, rinsed with PBS to remove culture medium, and then lysed by sonicating in a GITC lysis buffer. Placement of the vessel on ice while washing and lysing the cells will further protect the RNA from endogenous RNases released during the disruption process.

Soft, fresh plant tissue can often be disrupted by sonicating in a lysis buffer. Other plant tissues, like pine needles, need to be ground dry, without liquid nitrogen. Some hard, woody plant materials require freezing and grinding in liquid nitrogen prior to being ultrasonically processed. Plant cell suspension cultures and calluses can typically be sonicated in a lysis buffer within 2 minutes. The diversity of plants and plant tissue make it impossible to give a single recommendation for all. However, most plant tissues typically contain polysaccharides and polyphenols that can coprecipitate with RNA and inhibit downstream assays. Treating a plant tissue lysate with polyvinylpyrrolidone (PVP) will precipitate such problematic components from the lysate before the actual RNA isolation is carried out.

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. If sub-cellular particles are desired intact, the amplitude should be kept low, and the processing time increased.

Yeast can be extremely difficult to disrupt because their cell walls may form capsules or nearly indestructible spores. To process yeast, sonicate in a tube containing the sample, guanidinium-based lysis buffer and small glass beads (0.5 – 1 mm). Pretreatment with

zymolase, glucalase and / or lyticase to produce spheroplasts that are readily lysed may also be useful.

To disrupt filamentous fungi, scrape the mycelial mat into a cold mortar, add liquid nitrogen and grind to a fine powder with a pestle. The powder can then be thoroughly sonicated in lysis buffer to solubilize completely. As fungi may also be rich in polysaccharides, pretreatment with polyvinylpyrrolidone (PVP) may be beneficial.

Bacteria, like plants, are extremely diverse; therefore, it is difficult to make one recommendation for all bacteria. Ultrasonic processing will lyse most Gram positive and Gram negative bacteria, including mycobacteria. Although it is recommended that glass beads and lysis solution be used; it is possible to lyse some Gram negative bacteria by sonicating in lysis solution without beads. Bacteria cell walls can be digested with lysozyme to form spheroplasts. Gram positive bacteria usually require more rigorous digestion and longer processing time. The spheroplasts are then lysed with sonication in GITC lysis buffer.

Disruption of cells found in soil and sediments is accomplished one of two ways. One technique isolates the bacterial cells from the material prior to the RNA isolation procedure. This is accomplished by homogenization of wet soil in a mechanical blender followed by a slow speed centrifugation to remove fungal biomass and soil debris. The supernatant is centrifuged again at a higher speed to pellet the bacteria cells. Cells can then be lysed as described above for bacteria. Other techniques describe RNA isolation from the soil or sediment directly. For example, one method requires soil to be added to a diatomaceous earth and lysis buffer, and then sonicated. The sample is then centrifuged to remove solid debris.

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, N₂O, cysteine, reduced glutathione, dithiothreitol or other SH compounds, might be beneficial. Saturating the sample with a protective atmosphere of helium or nitrogen gas, or dropping a small pellet of dry ice in the sample, will also inhibit free radical formation. 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.

Various methods can be used to measure the efficiency of the disruption. For example, a visual count can be made using a microscope.

For greater accuracy, a protein assay could be used. This procedure is widely recognized as a good method for measuring cell disruption by taking into account the amount of protein released after disruption. The disrupted cells are then tested and checked against this number for percentage breakage.

There are several types of protein assays. One commonly used is the Folin Reaction (Lowry Assay) method, as it is comparatively simple and provides consistent results. This colorimetric method has a sensitivity to protein of around 8 µg / mL in the assay solution.

The assay turns blue in the presence of proteins due to the reaction of copper ions in the alkaline solution with protein and the reduction of phosphomolybdate- phosphotungstic acid in the Folin reagent by aromatic amino acids in the treated protein.

Fractional protein release, Rp, is calculated using the following equation and multiplying the result by 100:

$$Rp = \frac{Cf - Cb}{Ct - Cb}$$

Cf = Free protein

Ct = total protein

Cb = Background protein

This gives the actual disruption percentage, taking into account the background levels of protein before disruption.

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.

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 about contamination from previous use, clean the probe with a 20% Virkon solution and rinse with distilled water. Because Virkon®, Dupont's broad spectrum cleaner / disinfectant is effective against a wide range of virus, bacteria and fungi, it is ideally suited for that purpose. For further information regarding Virkon® contact BiosafetyUSA, Inc. 10220 NW 50th Street, Sunrise Florida 33351, 954-578-6580 *124 or e-mail info@biosafetyusa.com For critical application, probes may be autoclaved.

To inhibit 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.

High viscosity and concentration are problematic. 2,000 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 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, whenever possible, 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 to optimize cooling. Processing samples in a Cup Horn will usually take 4 times longer than processing with the direct probe intrusion method.