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USER'S GUIDE

HIGH INTENSITY ULTRASONIC PROCESSOR
Microprocessor Controlled
300-Watt Model ? 375-Watt Model
600-Watt Model
600-Watt Model Dual Output

TABLE OF CONTENTS

Important Safeguards
Low Surface Tension Liquids - Organic Solvents

SECTION 1 – INSTALLATION
Inspection
Electrical Requirements
Installing the Ultrasonic Processor

SECTION II – OPERATION
Principles of Ultrasonic Disruption
Functions of Controls, Indications, and Connectors
Preparations for Use
Tuning
Using the Ultrasonic Processor

SECTION III – SERVICE INFORMATION
Return of Equipment

SECTION IV – OPERATING SUGGESTIONS AND TECHNIQUES

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. 2003

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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.

When using a microtip, never allow the OUTPUT CONTROL setting to exceed MICROTIP LIMIT “5”.

Never operate a probe with threaded end without a tip, extender or microtip.

It is recommended that 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

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.



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 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. 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" (3 mm)	3/16" (5 mm)	1/4" (6.5 mm)	1/8" (3 mm)
INTENSITY	ultra high	very high	high	very high
VOLUME (batch)	1-10 ml	3-15 ml	5-25 ml	250ul-10 ml

STANDARD PROBES			
TIP DIAMETER	1/2" (13 mm)	3/4" (19 mm)	1" (25 mm)
INTENSITY	high	medium	low
VOLUME (batch)	10-250 ml	25-500 ml	50-1000 ml

HIGH GAIN PROBES		
TIP DIAMETER	3/4" (19 mm)	1" (25 mm)
INTENSITY	high	medium
VOLUME (batch)	25-500 ml	50-1000 ml

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FUNCTIONS OF KEYS, CONTROLS, INDICATIONS, AND CONNECTORS

FRONT PANEL	
POWER MONITOR (METER)	Indicates the percentage of maximum available ultrasonic power delivered to the probe. 300 watt unit: 100% = 300 watts, 50% = 150 watts. 375 watt unit: 100% = 375 watts, 50% = 187.5 watts. 600 watt unit*: 100% = 600 watts, 50% = 300 watts. * When using two probes with the dual output unit, the power delivered to each probe is half that displayed on the power monitor.
POWER SWITCH	ON position – energizes the power supply. OFF position – de-energizes the power supply. Illuminates when the power supply is energized.
TUNE SWITCH	When depressed, allows the power supply to be tuned.
TIMER	Sets the duration of ultrasonic application from .1 second to 999 hours. The right hand push button selects the timing option as follows: .S=tenth of seconds, S=seconds, .M=tenth of minutes, M= minutes .H=tenth of hours, H=hours. The other three push buttons select the timing increments
START BUTTON	When depressed, energizes the ultrasonic.
RESET BUTTON	When depressed, de-energizes the ultrasonic and resets the timer.
TUNER	Optimizes performance by matching the frequency of the power supply to that of the converter / probe assembly.
FOOTSWITCH CONNECTOR	Connects to footswitch cable.
PULSER	Applies the ultrasonic energy on a pulsed mode. In the OFF position the ultrasonic is continuous. In the ON position the ultrasonics is intermittent. The % DUTY CYCLE SELECTOR sets the pulse rate. 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.
OUTPUT CONTROL	Controls the amplitude of vibration at the probe tip. CAUTION *When using a microtip, never allow the OUTPUT CONTROL setting to exceed MICROTIP LIMIT "5".*

REAR PANEL	
3 PIN CONNECTOR (3)	Connects to the converter cable(s).
FUSE(S)	Protects against electrical overload
ELECTRICAL LINE CORD	Connects the power supply to the electrical outlet.

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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 POWER SWITCH is set to OFF.
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 2½" (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

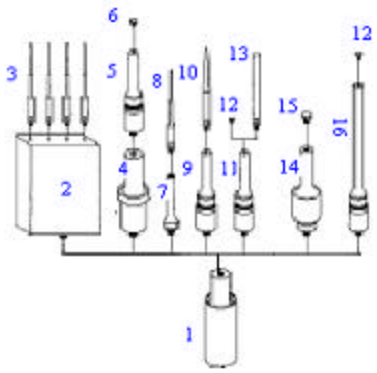


TIP REMOVAL



TIP TIGHTENING

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No	DESCRIPTION	Order Number
1	Converter Model CV17	CV00017
2	Four element coupler	630-0425
3	Stepped microtip 1/8" (3mm)	630-0422
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	Same as 5	
10	Tapered microtip $^{1}/_{8}$" (3mm)	630-0418
	Tapered microtip $^{3}/_{16}$" (5mm)	630-0419
	Tapered microtip ¼" (6mm)	630-0420
11	Same as 5	
12	Same as 6	
13	Extender ½" (13mm)	630-0410
	Extender ¾" (19mm)	630-0409
	Extender 1" (25mm)	630-0444
14	High gain probe ¾" (19mm) – solid	630-0306
	High gain probe 1" (25mm) – solid	630-0310
15	Same as 6	
16	Full wave ½" (13mm)- 10" (254 mm) long probe w/ replaceable tip	630-0218

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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TUNING

Tuning optimizes performance and insures maximum transfer of energy by matching the frequency of the power supply to that of converter/probe assembly. The power supply should be tuned 1) every time a new probe or accessory is used, 2) on occasions to compensate for the frequency variation caused by cavitation erosion 3) following 10 minutes of continuous operation and 4) when the sample temperature is significantly higher or lower than room temperature.
The piezoelectric crystal within the converter is part of the circuitry, which controls the frequency at 20kHz. Any changes in t the crystal's capacitance resulting from a variation in temperature, will cause the equipment to operate in an out-of-tune condition. For reliable performance, and equipment protection, it is important that the unit be tuned after the probe temperature has had a chance to stabilize. When relocating the ultrasonic processor from a very cold or very hot environment, allow 30 minutes for the unit temperature to stabilize before operating. Continuous operation causes temperature elevation in the sample. This increase in temperature is transmitted through the probe to the crystal assembly. Always tune the power supply after the probe has reached operating temperature. When working with low or high temperature samples, immerse the probe in the sample for a few minutes, withdraw the probe from the sample, then tune the power supply.

IMPORTANT
Tuning must be performed in air with the probe out of the sample. While tuning, do not allow the probe to contact anything.

1. Ensure that the probe or microtip is not immersed in the sample and that it does not come in contact with anything. If a cup horn is used, make sure that the water has been drained out of it. If a flow through cell is used, make sure that the sample has been drained out of it.

2. Set PULSER to OFF.

3. SET output control to "10" (to "5" when using a microtip of extender).

CAUTION
When using a microtip, never allow the tip to vibrate in air for more than 10 seconds and do not set the OUTPUT CONTROL above "5". Ignoring these instructions will cause the microtip to fracture.

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4. Set POWER SWITCH to ON. The switch will illuminate.
5. **Momentarily** depress TUNE SWITCH and rotate the tuner clockwise or counterclockwise until a **minimum** (not maximum) reading (usually less than 20) is obtained on the POWER MONITOR. If a minimum reading (sometimes referred to as null) cannot be obtained, the probe, cup horn, tip, microtip, or accessory is loose or out of resonance, or the power supply or converter require servicing. A loose probe will usually generate a loud piercing sound.

NOTE

If minimum reading cannot be obtained, check unit without the probe to ascertain whether the power supply or probe is at fault.

6. Set OUTPUT CONTROL to “4”.

7. Set POWER SWITCH to OFF.

8. With a dual output 600 watt Ultrasonic Processor, if two converters are going to be used simultaneously, connect the second converter cable to the top connector.

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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 "5". 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.

OPERATING INSTRUCTIONS

1. Ensure that the power supply is properly tuned. Tuning optimizes performance and insures maximum transfer of energy by matching the frequency of the power supply to that of the converter / probe assembly
2. If a standard probe is used, immerse the probe approximately 2 inches (5 cm) into the sample. If a microtip is used, immerse the microtip approximately ½" (1 cm) into the sample.

NOTE

The probe should be immersed to a sufficient depth to preclude air from being injected into the sample, and inhibit aerosoling and foaming.

3. Set TIMER as required. If the footswitch is used, set TIMER to 999 hours. Depressing the footswitch will not energize the power supply, unless the TIMER is activated.

4. Set PULSER as required. If the pulsing mode is not required, set PULSER to OFF.

5. Set POWER SWITCH to ON. The switch will illuminate.

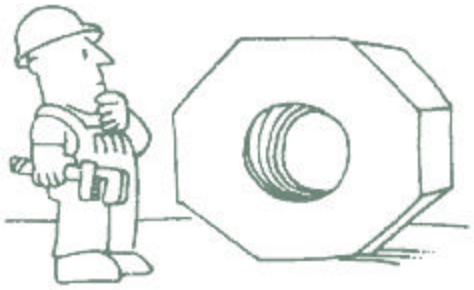
6. Activate the timer by depressing the START button.

7. If the footswitch is used, depress footswitch.

8. Using the OUTPUT CONTROL increase or decrease the intensity as required

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SECTION IV – 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. Replace the fuse(s).
3. Set the OUTPUT CONTROL to “10” 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.
4. 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.
5. If the Ultrasonic Processor stops working investigate and remedy the problem.

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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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SECTION V - 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 of 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.

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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 that 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 that 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 37ºC, a week at 25ºC a month at 4ºC 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, byaluronidase, 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. Collogenase may be used with collogen, lysostaphin with staphylococcus, and trypsin hyaluronidase with liver and kidney.

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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, undisrupted. 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

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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, N_2O, cysteine, reduced glutahione, 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.

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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:

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

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. For critical application, probes may be autoclaved.

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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.

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