USER'S GUIDE

HIGH INTENSITY ULTRASONIC PROCESSOR

MODEL VCX 100 SA

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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.

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.

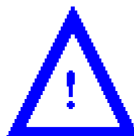
IMPORTANT SAFEGUARDS

READ BEFORE INSTALLING OR

USING THE EQUIPMENT

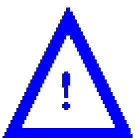
Your Ultrasonic Processor has been designed with safety in mind. However, no design can completely protect against improper usage, which may result in bodily injury and/or property damage. For your protection and equipment safeguard, observe the following warnings at all times, read the operating instructions 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.

- High voltage is present inside the unit. Do not remove the bottom cover. Refer all servicing to qualified service personnel.
- Never touch a vibrating probe.



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.



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 phrase 3-terminal grounding type electrical outlet capable of supplying 100 – 240 VAC.

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.

SECTION II – OPERATION

NOTE

The Ultrasonic Processor is available with four probes – a 2mm ($^{5}/_{64}$") microtip, a 3mm ($^{1}/_{8}$") microtip, a 6mm ($^{1}/_{4}$") microtip, a 13mm ($^{1}/_{2}$") probe, and a 13mm (½") probe with replaceable tip.

- The 2mm microtip is optional, and can process between 0.2 ml and 5 ml.
- The 3mm microtip is standard, and can process between 0.5 ml and 10 ml.
- The 6mm microtip is optional and can process between 5 and 50 ml.
- The 13mm probe is optional, and can process between 50 and 150 ml.

DISPLAY AND CONNECTOR FUNCTIONS

FRONT	
LCD screen	Displays prompts and the following control parameters: • Amplitude selected • Amount of output power delivered to the probe in watts, and as a percentage of 100 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.
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 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.
I 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 the selected amplitude, then depress the ▲ or ▼ key as required.

REAR	
Coax connector	Connects to the converter.
Electrical connector	Connects to the 24 Volt adapter.

PREPARATION FOR USE

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

1. Plug the electrical adapter into the electrical outlet and the electrical connector at the rear of the enclosure. If the unit is already on; depress the O key.

2. If the converter / probe assembly is not already assembled, screw the probe into the converter and secure tightly with the two wrenches provided. Do not secure using only one wrench.

3. Place the converter / probe assembly in the stand.

4. Feed the converter cable through the opening at the top of the enclosure, and connect it to the coax connector at the rear of the enclosure.

> **NOTE**
> Should it become necessary to remove a probe from the converter, always use two wrenches. Do not remove using only one wrench.

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 fully clockwise will not cause the maximum power to be delivered to the sample. The maximum power (100 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.

CAUTION

- Do not operate the unit unless the converter is connected to the coax connector.
- Do not allow the probe to vibrate in air for more than 10 seconds.
- Do not allow the vibrating probe to contact anything but the sample.

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 4 and 0 for a 40% reading on the screen – Ampl 40%, then press 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, 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.

Immerse the probe halfway 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 being processed, it is recommended that the intensity be selected empirically, by increasing or decreasing the amplitude 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 10 minutes processing time, the elapsed time will be 20 minutes 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: 00 Sec: 30

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

TIME 0:00:30
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 1 second to 59 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:

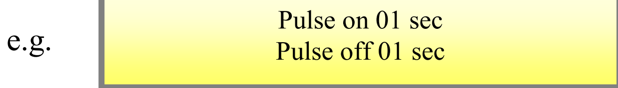
e.g.

Pulse on 01 sec
Pulse off __.__sec

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 0:00:30 Time 0:22:10
c) Selected pulsing cycle, and actual pulsing cycle:
   e.g. Pulse 2.0 1 / 1
d) Amount of power in watts, and accumulated amount of energy in JOULES delivered to the probe:
   e.g. 25 watts 0001265 Joules
e) Elapsed time since processing was initiated:
   e.g. Elapsed time 0:04:20

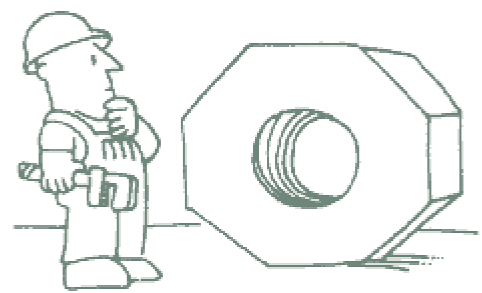
NOTE

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 10 hours of 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 15 watts with the probe ***out of the sample***, when the AMPLITUDE is set at 100. If the wattmeter reads over 15 watts, the probe 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 probe is not secured properly, is too eroded, or is fractured.

1. Press the I key and set the amplitude to 100. With the probe in air (***out of the sample***), the wattmeter should read below 15 watts. If the reading exceeds 15 watts, press the O key, and using two wrenches, disconnect the probe from the converter.

2. Press the I key. If the wattmeter reads below 15 watts, the probe has failed or is out of tune due to excessive erosion, or fracturing, and should be replaced, if the wattmeter reads above 15 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 above paragraph, then press the O key to switch the unit off, and the I 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 CERTICATION CAN BE MADE.

SECTION IV – OPERATING SUGGESTIONS AND TECHNIQUES

ABOUT ULTRASONICS

The ultrasonic power supply (generator) converts 50/60 Hz voltage to high frequency electrical energy. This alternating voltage is applied to disc-shaped ceramic piezoelectric crystals within the converter, causing them to expand and contract with each change of polarity. These high-frequency mechanical longitudinal vibrations are amplified by the probe (horn) and transmitted into the liquid as alternating expansive and compressive acoustic waves. The pressure fluctuations cause the liquid molecule cohesive forces to break-down, pulling apart the liquid and creating millions of micro-bubbles (cavities), which expand during the low pressure phases, and implode violently during the high pressure phases. As the bubbles collapse, millions of microscopic shock waves, micro jet streams, eddies, and extremes in pressures and temperatures are generated at the implosion sites and propagated to the surrounding medium. Although this phenomenon, known as cavitation, (sometimes referred to as cold boiling), lasts but a few microseconds, and the amount of energy released by each imploding bubble is minimal, the cumulative amount of shear force(s) generated is (are) extremely high.

The intensity of cavitation that takes place in a liquid is proportional to the intensity of the vibrations at the probe tip (amplitude), the surface tension and temperature of the liquid, the viscosity, and the pressure that the liquid is being subjected to. In general, the greater the amplitude of oscillation at the tip of the probe, the higher the level of cavitation. Viscosity and surface tension greatly affects cavitation and the higher the viscosity or surface tension of the liquid, the greater the acoustic attenuation which will dampen the cavitation process and the greater the amount of energy that will be required to cavitate the liquid. Highly viscous liquids are “sluggish” and cannot respond quickly enough to form cavitation bubbles and violent implosion. Temperature of the liquid being ultrasonicated has a great influence on the intensity of cavitation. Temperature modifies several properties of the liquid medium, such as viscosity, surface tension, and mainly vapor pressure, all of which influence cavitation.

Although elevated temperature weakens bubble collapse due to high vapor pressure, the higher the temperature, the higher the number of cavitation bubbles. Cavitation increases up to 70°C and then decreases and stops completely at the boiling temperature of the liquid. For most applications the temperature should be kept between 40°F and 70°F.

A pressurized liquid will require more ultrasonic energy (higher amplitude) to produce cavitation, and the greater the pressure, the greater the amount of energy that will be released into the medium as the bubbles implode.

Ultrasonic probes

Probes, (sometimes referred to as sonotrodes or horns), are one-half wavelength long tools that act as mechanical transformers to increase the amplitude of vibration generated

by the converter. They consist of two sections each having different cross-sectional areas. When driven at its resonant frequency, the probe expands and contracts longitudinally about its center. However, no longitudinal motion occurs at the threaded nodal point (area of no activity), allowing accessories to be connected to the probe at that point. The greater the mass ratio between the upper section of a probe and the lower section, the greater the power magnification/amplification factor, and the greater the peak-to-peak excursion at the tip of the probe. Probes with smaller tip diameters produce greater intensity of cavitation, but the energy released is restricted to a narrower, more concentrated field. Conversely, probes with larger tip diameters produce less intensity, but the energy is released over a greater area. A multitude of probes are available for a given range of volumes, and mistakes such as using small diameter probes for high volume processing, or large diameter probes for small volume processing should be avoided. The larger the tip diameter, the larger the volume that can be processed, but the processing occurs at a lower intensity. Probes are fabricated from high grade titanium alloy Ti-6Al-4V because of its high tensile strength, good acoustical properties at ultrasonic frequencies, high resistance to corrosion, low toxicity, and excellent resistance to cavitation erosion. They are autoclavable and available with threaded ends to accept replaceable tips and microtips.

NOTE:

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 the correct frequency, and the power supply will go into an overload condition and stop working or fail. Unlike aqueous (water based) solutions, which rarely cause problems, solvents and other low surface tension liquids are problematic. These liquids penetrate the probe/replaceable tip interface, and force particulates into the threaded section isolating the tip from the probe.

The size of the probe best suited for an application is dependent on the volume of liquid to be processed. Microtips should never be used to process samples larger than 50 ml.

When processing low surface tension liquids ALWAYS use a solid probe.

Temperature

During sonication, processing will typically cause the temperature of the liquid to increase, especially with small volumes. Excessive heating can cause substantial liquid evaporation, resulting in changes to the sonicated volume, or the degradation of the material or medium components.

A simple approach to minimize temperature driven side effects is to avoid substantial high-temperature excursions by immersing the suspension container in an ice bath, (the container should be immersed to a level roughly equal to that of the internal suspension), and by pulsing the ultrasonics on and off. While processing the sample, occasionally touch the vessel to ensure that the sample is relatively cool.

Ultrasonic processors can typically operate in either continuous or pulsed mode. In pulsed mode, ultrasonic intervals are alternated with static (sonication off) intervals. The duration of on and off intervals can be regulated. Operating in pulsed mode retards the rate of temperature increase in the medium, minimizing unwanted side effects and allowing better temperature control than continuous mode operation. Short pulse mode operation interspersed with cooling periods is therefore generally recommended. When working with small volumes the temperatures of the suspension will rise more rapidly; therefore, more intense cooling conditions may be required.

Working with containers made of materials with high thermal conductivities helps ensure a rapid release of heat from the suspension. The following container materials are suggested, in order of decreasing thermal conductivity: stainless steel, glass, plastic. When selecting the container, consideration must also be given to the chemical compatibility between the container material and the suspension components. For instance, glass is incompatible with alkaline solutions. If the analyte under investigation is a phthalate, then plastic containers should be avoided.

An aluminum foil or other cover with an opening just big enough to allow the probe to pass through is recommended to reduce evaporative loss of liquid content, especially when working with volatile solvents (e.g., ethanol) or when processing the liquid for prolonged durations. Cooling the sample will inhibit evaporation and using a cover will also minimize the potential release of aerosols generated by the sonication process.

Processing time and concentration

The total amount of energy (E) delivered to a liquid not only depends on the applied power (P) but also on the total amount of time (t) that the liquid is subject to the ultrasonic treatment: E=P x t. Two liquids treated at the same power level for different times will show significantly different dispersion rates.

While the amount of power delivered, and the duration of processing translates into the amount of energy delivered to the liquid, samples of different volumes and particle concentrations will respond differently to the same amount of energy.

In general, processing a liquid at a low intensity for a long period of time, will give the same results as processing for a short period of time at high intensity. Processing high particle concentration liquids for long durations will result with an increase in particle collision frequency, causing particle size reduction due to abrasion and breakage. Results are thus dependent on both the level of energy delivered and the physiochemical properties of the suspension. Rather than using high energy, it is best to select the lowest amplitude that will give satisfactory results, as over-processing may cause sintering and re-agglomeration. When performing particle size analysis, one should remember that when processing friable materials the intent is to disperse and not to fracture the particles. It’s only after treating the samples with varying intensity and duration, and performing

frequent analysis (about every 20 seconds) using a microscope, that one will be able to determine the optimum processing parameters.

Ultrasonic probe, container geometry, and tip immersion

At equivalent instrumental power settings, microtips vibrate with greater amplitudes than conventional bigger diameter probes. However, microtips are less mechanically robust and are limited in terms of the maximum power setting at which they can be used. Driving a microtip at too high of an amplitude will cause the tensile strength of the titanium probe to be exceeded, and the microtip to fracture. Microtips are recommended when processing small suspension volumes (e.g., 10ml or less).

The way in which the ultrasonic energy is distributed within the suspension is also heavily influenced by the container geometry. When possible, it is recommended to use the smallest diameter vessel that allows for the probe to be inserted without touching the container walls. Using smaller container diameters raises the height of the liquid and maximizes the liquid-probe surface area exposed to the acoustic waves.

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, with the space between the probe and the walls of a vessel being kept to a minimum as the energy rapidly decreases both radially and axially as the distance from the probe increases. 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.

The probe should not be allowed to touch the bottom or the side of the vessel. 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. Microtips must never 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.

Probe immersion depths halfway from the bottom and the top of the liquid is usually the placement of choice as to where the tip should be located. Probes should be placed no closer than about 1” (13mm) from the bottom of the container.

Aerosoling and foaming

When processing a sample with ultrasonics, always immerse the probe deep enough below the surface of the sample to inhibit aerosoling or foaming, as foaming substantially reduces cavitation. When lysing cells the probe should not be immersed too deeply as then proper lysis will not take place. 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.

If the sonicator probe is not sufficiently immersed in the liquid it can give rise to surface agitation resulting in nebulization (formation and release of aerosols). This could pose a risk if harmful medium components are released in this manner.

Aerosoling may be indicated by changes in the audible sound pitch during operation, fluctuating power readings, or by the appearance of a fine aerosol in the vicinity of the probe. If aerosoling occurs the probe should be immersed deeper into the liquid.

If surfactants are used, the liquid could foam during sonication, interfering with the delivery of ultrasonic energy into the liquid. Pulse mode operation with long off periods will inhibit foaming in samples subject to this effect. Sonication should be terminated in the event that foaming occurs, and the foam removed or allowed to dissipate before continuing.

Disrupting cells/Cell lysis

Sonication is commonly used in the fields of molecular biology and proteomics, because the extremely powerful forces generated by the cavitation are capable of rapidly disrupting cell walls and facilitating access to intracellular pathogen-identifying material.

In animal cells, the plasma membrane is the only barrier separating cell contents from the environment, but in plants the plasma membrane is also surrounded by a rigid cell wall. The lack of an extracellular wall in animal cells makes them relatively easy to lyse. Plant cell walls consist of multiple layers of cellulose. These types of extracellular barrier confer shape and rigidity to the cells. Plant cell walls are particularly tough, making them very difficult to disrupt mechanically or chemically.

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 can be lysed by sonication in a lysis buffer for 30 seconds to 2 minutes. The diversity of plants and plant tissue make it impossible to give a single recommendation for all samples. However, one should be aware that 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.

When processing plant tissues, it is important to remember that plants have periods of active metabolism where harvesting and storage conditions will impact the levels and

conditions of analytes. For instance, during the germination of seeds, cells not only experience rapid growth, but they are also adjusting to environmental stimuli. Older samples will have a very different RNA profile than freshly collected and homogenized samples. However, with seeds that are dried and relatively dormant, the collection and homogenization process probably has a much smaller impact on the levels and condition or analytes.

Single-cell organisms (micro-organisms) consist of a semi-permeable, 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 intracellular 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. 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.

Sonicating an organism to liberate its genomic material is an important step in sample preparation for nucleic acid testing. Many common pathogens can be lysed through chemical agents, such as detergents and chaotropic salts, or by enzymatic treatment. However, these methods present a significant challenge when processing thick-walled microorganisms such as Bacillus anthracis spores and Mycobacterium tuberculosis cells. The multilayer structure of Bacillus spores includes an outer cortex and coat that is resistant to chemical and physical treatments. Similarly, mycobacteria have a thick, waxy cell wall that is difficult to disrupt for the extraction of nucleic acids.

Sonication is ideally suited for processing these thick-walled organisms, since chemical, heat, freeze-thaw, or enzymatic lysis methods alone are not very effective.

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.

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. Bacteria 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. Gram positive bacteria usually require more rigorous digestion than Gram negative organisms. Bacteria cell walls can be digested with lysozyme to form spheroplasts. The spheroplasts are then easily lysed in a GITC lysis buffer with ultrasonics.

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

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. In general, DNA extraction requires softer sonication.

The radiated energy will cause the sample to heat up. In order to prevent protein degradation always keep the sample ice cold, before, during, and after processing. When processing heat sensitive samples, short burst pulsing is recommended, (especially when using a microtip), 5 seconds on and 5 seconds off.

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.

NOTE:

All living organisms contain proteolytic enzymes (proteases and peptidases). Proteases are required for a variety of cellular functions, such as cellular repair or the digestion of extracellular material. In whole cells, protease activity is tightly regulated by compartmentalization or inhibitors to prevent damage to cellular proteins. Cell lysis disturbs this regulation and proteolytic degradation of the sample becomes a concern. Therefore, addition of protease inhibitors to cell lysis buffers is often required. Protease inhibitors are biological or chemical compounds that functions by reversibly or irreversibly binding to the protease. Proteases generally belong to one of four evolutionarily distinct enzyme families based on the functional groups involved in cleavage of the peptide bond. Therefore, several different types of inhibitors are generally required to protect proteins from proteolysis during extraction and purification.

The disruption of samples is an early step in the process of isolation and/or quantifying RNA, DNA, proteins, and analytes. Ultrasonic degradation of DNA in solution occurs by breaking hydrogen bonds and by single-strand and double-strand ruptures of the DNA helix. Following sonication, the distribution of the resulting DNA fragments typically approaches a lower size limit of 100 – 500 bp. The relative lack of specificity in degrading the DNA helix makes ultrasonication a complementary alternative to the highly specific fragmentation obtained by restriction endonucleases.

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. For most samples, thorough disruption can be monitored by close

inspection of lysate after disruption. There should be no visible particulates, except when disrupting materials containing hard, non-cellular components, such as connective tissue or bone.

Prior to sonication, difficult cells can be pre-treated with various agents to aid the disruption process. Lysis can be promoted by suspending cells in a hypotonic buffer, which causes them to swell and burst more readily by physical shearing. Lysozyme can be used to digest the polysaccharide component of yeast and bacterial cell walls. This treatment is commonly used with yeast cells. Viscosity of a sample typically increases during lysis due to the release of nucleic acid material. DNase can be added to samples (25-50 μg/ml) to reduce this problem. Nuclease treatment is not required for sonicated material because sonication shears chromosomes. Because proteolysis can be a problem whenever cells are manipulated; it is advisable to add protease inhibitors to samples undergoing lysis. It is common that a protease inhibitor is added to lysis buffer, along with other enzyme inhibitors of choice, such as a phosphotase inhibitor when studying proteins with phosphorylation. Lysozyme, byaluronidase, glycosidase, glucalase, lyticase, zymolase and lysostaphin digestion are among the enzymatic methods frequently used with yeast and 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.

The initial size of the particles to be disrupted is an important factor when using ultrasonics for processing. Hard tissues such as muscle, liver, heart, kidney, spleen, and lung have all been successfully disrupted after they have first been processed in an upstream disruption such as blending, snap freezing, or mechanical homogenization. The lysate from a two-step homogenization process of tissue, (i.e. grinding then sonication), results in superior liberation of enzymes and analytes than grinding or sonication does alone.

For microorganisms, the addition of glass beads in the 0.050 mm (50 microns) – 0.1 mm (100 microns) diameter range will expedite cell disruption as it facilitates the crushing of the cell walls. Beads are almost a prerequisite when working with spores and yeast. A good ratio is one volume of beads to two volumes of liquid.

Yeast can be extremely difficult to disrupt. When processing yeast, it is recommended that guanidinium-based lysis buffer, and small glass beads be added to the sample to be processed.

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

Disruption of cells found in soil and sediments is accomplished by 1) isolating 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 to pellet the bacteria cells. From this point, cells can be lysed as described above for bacteria, or 2) isolating RNA from the soil or sediment directly; for example, adding soil to a diatomaceous earth and lysis buffer, and then sonicating. The sample is then centrifuged to remove solid debris.

Cultured cells are relatively easy to disrupt. Cells grown in suspension are collected by centrifugation, rinsed to remove culture medium, and then lysed by sonicating in GITC lysis buffer. Placing the processing vessel on ice while washing and lysing the cells will further protect the RNA from endogenous Rnases released during the disruption process.

To expedite disruption when using ultrasonics, cells can be treated with some weak detergent such as digitonin, before being exposed to ultrasonics. Detergents break the lipid barrier surrounding cells by solubilizing proteins and disrupting lipid:lipid, protein:protein and protein:lipid interactions. When the goal of cell lysis is to purify or test the function of a particular protein, special attention must be given to the effects of the lysis reagents on the stability and function of the protein(s) of interest. Certain detergents will inactivate the function of particular enzymes, and long-term stability of extracted/purified proteins often requires that they be removed from the initial lysis reagents and/or stabilized by addition of particular compounds. One should keep in mind that although useful for nucleic acid isolation, detergents may denature proteins which can make their use undesirable in protein purification schemes. The same is true for the addition of lytic enzymes, which in the case of protein purification, must be subsequently removed.

Unfortunately, there is no standard protocol available for selecting a detergent to use for membrane lysis, the ideal detergent will depend on the intended application. In general, nonionic and zwitterionic detergents are milder and less denaturing than ionic detergents and are used to solubilize membrane proteins when it is critical to maintain protein function and/or retain native protein:protein interactions for enzyme assays or immunoassays. Zwitterionic detergents and nonionic detergents are commonly used for these purposes. In contrast, ionic detergents are strong solubilizing agents and tend to denature proteins, thereby destroying protein activity and function. There are a few commonly used ionic detergents that are only mildly denaturing, including sodium cholate and sodium deoxycholate. Nonionic detergents like Triton X-100 and zwitterionic detergents like CHAPS (3[3-cholamidopropyl) dimethylammonio] -1-propanesulfonate) are nondenaturing (will not disrupt protein functions). While ionic detergents like sodium dodecyl sulfate (SDS) and cationic detergents like ethyl trimethyl ammonium bromide are denaturing (will disrupt protein functions). Detergent dosage should be kept to a minimum, as it has the undesirable effect of contaminating the preparation.

The choice of detergent for cell lysis also depends on sample type. Animal cells, bacteria and yeast all have differing requirements for optimal lysis due to the presence of absence of a cell wall. Because of the dense and complex nature of animal tissues, they require both detergent and sonication. In addition to the choice of detergent, other important considerations for optimal cell lysis include the buffer, pH, salt concentration and temperature. Consideration should be given to the compatibility of the chosen detergent

with downstream applications. For example, if the detergent used for lysis must be removed, then a dialyzable detergent should be selected.

If pretreatment with enzymes or detergents cannot be used, the freeze / thaw method should be considered. The freeze / thaw method is commonly used prior to lysing bacterial and mammalian cells. This technique, which should be used only when working with cellular components that are insensitive to temperature, involves freezing a cell suspension for 10 minutes in a dry ice and isopropanol bath and then thawing the material at room temperature immediately prior to ultrasonic processing. The freeze / thaw cycle should be repeated three times prior to being subjected to the ultrasonics. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing. This pretreatment has been shown to effectively release recombinant proteins located in the cytoplasm of bacteria and is recommended when lysing mammalian cells. However, this method is not recommended when working with nitrilases as it has been noticed that purified nitrilases suffer structural damages upon freezing.

Most animal tissues can be processed fresh (unfrozen). However, it is important to keep them cold and to process them quickly (within 30 minutes) after dissection. When disrupting fresh tissue, the cells need to be sheared 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.

Animal tissues that have been frozen after collection should be pre-treated 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 sonication in a GITC lysis buffer. Processing frozen tissue in this way is cumbersome and time consuming, but effective.

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 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 into 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.

Before each application, sonicate the tip in ethanol and for a few seconds to remove any residual substances. If still concerned about contamination from previous use, clean the probe with a disinfectant such as 20% Virkon solution and rinse with distilled water. Probes are autoclavable. 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.

The cup horn can process materials in complete isolation. Although in principle, similar to ultrasonic baths, they generate much greater intensity, and are commonly used for processing dangerous pathogenic, radioactive, biohazardous and volatile materials, and when concerned with aerosoling, cross-contamination and sterility. Because plastic tubes have a tendency to absorb vibrations, it is preferable to contain the sample in glass tubes when working with a cup horn. To expedite processing, add glass beads to the sample. If desired, in order to optimize cooling, crushed ice can also be added to the water inside the cup. Processing samples in a cup horn will usually require 4 times longer than processing with a probe.

Various methods can be implemented to measure the efficiency of ultrasonic disruption. Typically, counting the cells using a microscope is a satisfactory method. However, for greater accuracy, a protein assay can 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.

Typical cell lysis by sonication

1. Set the amplitude on the power supply to 30%.
2. Transfer the culture medium into a clean 15ml centrifuge tube, and centrifuge the contents at 3,200xg , 4 degree C for 5 minutes.
3. Without disturbing the pellets, aspirate 6ml of the supernatant into a clean centrifuge tube.
4. Add 2ml of suitable pH 7.5 buffer with protease inhibitors, and mix well using a Vortex mixer.
5. Incubate the sample on crushed ice for 40 minutes.
6. Insert the 1/8" (3mm) microtip half way inside the tube - not near the sample-air interface.
7. Keeping the cell suspension cold to prevent the degradation of the protein, sonicate with 10 bursts of 5 seconds (on cycle) followed by cooling intervals of 20 seconds (off cycle). If necessary, repeat. The viscosity of the sample will decrease due to macromolecular shearing. This action will cause the lysate to become clear and less viscous.
8. Incubate the lysate on crushed ice for 10 minutes.
9. Centrifuge out the cell debris at 30,000xg, 4 degree C for 20 minutes.
10. Transfer to a clean tube and store at -20 degrees C for future use. Avoid multiple freeze/thaw cycles.

Note: Protein concentration can be determined by the bicinchoninic acid method (Pierce protein assay 23228)

There are several types of protein assays. The most common is the Folin Reaction (Lowry Assay) method, as it is comparatively simple and provides consistent results.

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.

Hints and Tips

1. Always keep your samples on ice. The energy from the sonicator which causes your sample to break apart also heats it up. If your sample gets too hot the protein will start to degrade. In order to prevent this try to keep your sample on ice at all times, before, after and during the process if possible.
2. Reduce the temperature of your sample by pulsing. Pulsing reduces the heating up of your sample during sonication.
3. Do not over-Sonicate. Sonicating your sample for too long can degrade your protein. Finding that perfect balance may take some optimization and can vary for different cell/tissue types and sample volumes.
4. Sample volume and Probe size. The probe you use can vary depending on your sample size. Each probe has a recommended sample volume range. Small tips (microtips) are recommended for processing samples inside small thin vessels and never samples over 50ml. Microtips are made for short processing times. Microtips will generate considerable amount of heat in small volumes and should be used in the pulse mode to prevent heat build-up.
5. Processing a sample at a lower amplitude for long duration, will give better results than processing at a high amplitude for a short duration.
6. Sonicating at lower amplitude for longer duration will reduce heating of the sample. Amplitude and intensity have a direct relationship. In order to be able to reproduce results, the amplitude setting, temperature, viscosity and volume of the sample are all parameters that need to remain consistent. The amplitude is most critical when trying to reproduce sonication results.
7. Always clean the sonicator tip between samples. Cleaning the sonicator tip is critical in limiting protein carryover. Wiping the probe with 70% ethanol or processing ethanol in a beaker is an effective way of cleaning the probe.

Degassing a liquid

Degassing is usually performed by allowing the liquid to stand for some time before it is used so that the bubbles rise to the surface by their own buoyancy, however, in the case

of viscous liquids, the process is with most applications, too slow, thus the need for ultrasonics. Ultrasonic degassing is fast, and can be used to process liquids on a batch as well as on a flow-through basis.

When an ultrasonic probe is immersed in a liquid, the longitudinal vibrations radiated out from the tip are transmitted into the liquid as alternative high and low pressure waves. The pressure fluctuations cause the liquid molecules' cohesive forces to break down, pulling apart the liquid and creating millions of micro-vacuum bubbles (cavities) during the low pressure phases. The entrapped dissolved gases in the liquid permeate though the micro-bubbles outer surface, and as these gas bubbles pulsate in the ultrasonic field, they are propelled toward each other, causing them to collide into adjacent bubbles, coalesce and form larger bubbles. This growth accelerates until buoyancy is reached causing them to rise to the surface.

When using ultrasonics to degass a liquid, it is important to cycle the ultrasonics on and off, with the duration of the off-cycle being four times longer than the on-cycle (e.g. 5 seconds on and 20 seconds off / 1 minute on and 4 min off). The higher the viscosity the longer the on and off cycles. The longer off-cycle is necessary to allow the outgassed bubbles adequate time to rise to the surface, especially when processing viscous materials. To accelerate the process, preference should be given to using a shallow vessel over a tall vessel. If concerned about temperature elevation, consideration should be given to using an ice bath.