

# Cup Horn Manual

**Model #431C2**

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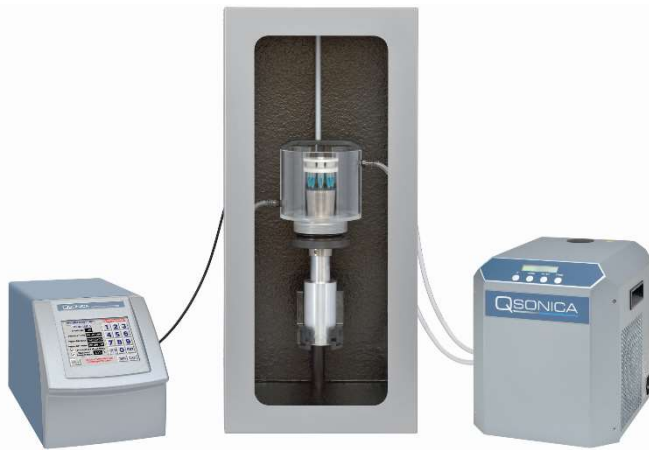
Rev. 3-24

## **Overview**

Cup Horns offer indirect sonication and function as high intensity ultrasonic water baths. Multiple samples can be processed in sealed tubes or vials to prevent aerosolization and cross contamination. Cup Horns are ideal for sterile or pathogenic samples processing.

The horn is mounted within an acrylic cup filled with water. Sample tubes are placed in a rack just above the horn. Cavitation is produced in the water, processing the samples within the tubes or vials. A coolant port is located on each side of the cup to circulate cold water and keep samples from overheating. A microtube rack and holder are included with each Cup Horn.

The Sound Enclosure (#432B2) is highly recommended for all Cup Horn users. In addition to reducing sonication noise to safe levels, it secures the Cup Horn in the proper position. The Sound Enclosure has ports on each side for coolant tubing to pass from the Cup Horn to an external water source or pump system. Please contact Qsonica for information on the Recirculating Chiller (Part# 4905) option.



**Q700, Sound Enclosure, Cup Horn & Chiller**



**#431C2**

The Cup Horn includes a sample tube holder (#440) for 8 polystyrene tubes (1.5mL). The unit is shipped with a set of polystyrene tubes, which are proven to transmit ultrasonic energy more efficiently than standard polypropylene tubes. Polystyrene tubes have large caps, so they require this specific tube rack.

**Note: Optimal results are achieved by using 0.2 or 0.5mL thin-walled PCR tubes or 1.5mL polystyrene tubes.**

Standard 1.5mL polypropylene tubes (Eppendorf type) are thick and block some ultrasonic energy from being transmitted into the contents of the tube. 1.5mL polypropylene tubes work well for many common applications. Use the #451 tube holder for these tubes. Certain samples that are difficult to lyse, such as yeast, tissues, or chromatin fixed with formaldehyde, require the 1.5mL polystyrene or smaller pcr tubes. 0.5mL pcr tubes work best for most applications.

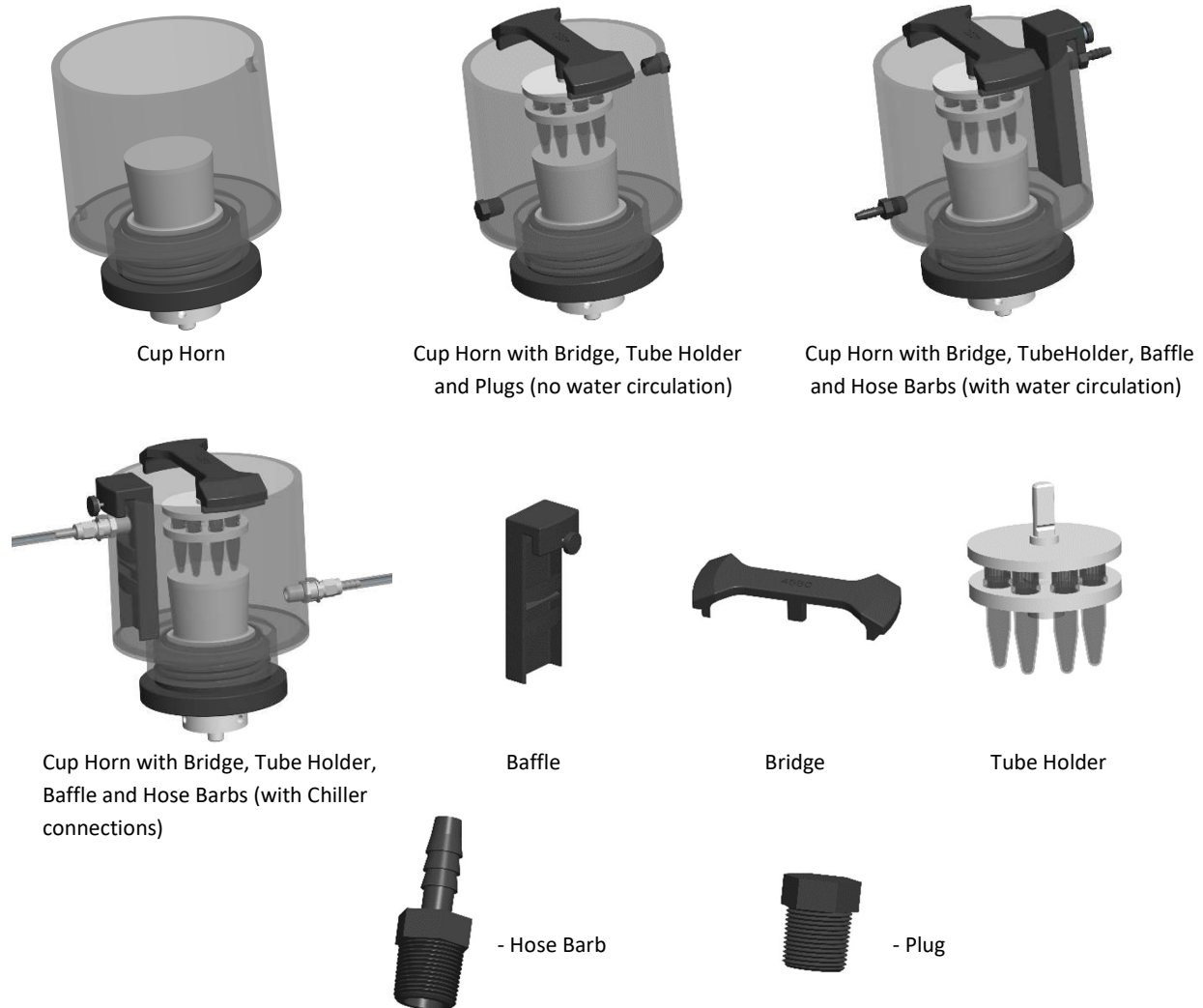
The Cup Horn is shipped with the following parts:

- Acrylic cup
- Titanium horn
- Threaded bushing (black)
- O-rings (2)
- #440 and #451 tube holders
- Bridge to hold Tube Holder inside Cup Horn
- Hose arb fittings (2)
- Plugs (2)

The customer installs the Plugs or Hose Barbs, depending on how they intend to cool the water inside the cup. Circulating cold water through the Cup Horn keeps samples cold and allows for shorter processing time. Without circulation, the horn heats quickly and may require pulsing ultrasonics on and off.

The Cup Horn is shipped with two tube racks for 1.5mL tubes and the Bridge to hold the tube racks. The chiller (#4905) and tubing/connector set (#4911) are optional. A Baffle is included with the #4911 set to direct a smooth flow of water through the system. The Baffle shown below is used with a Qsonica chiller and is not included with the Cup Horn.

If a Chiller is not used, the Cup Horn can be set up with basic hose barb fittings or plugs.



Cup Horn

Cup Horn with Bridge, Tube Holder and Plugs (no water circulation)

Cup Horn with Bridge, TubeHolder, Baffle and Hose Barbs (with water circulation)

Cup Horn with Bridge, Tube Holder, Baffle and Hose Barbs (with Chiller connections)

Baffle

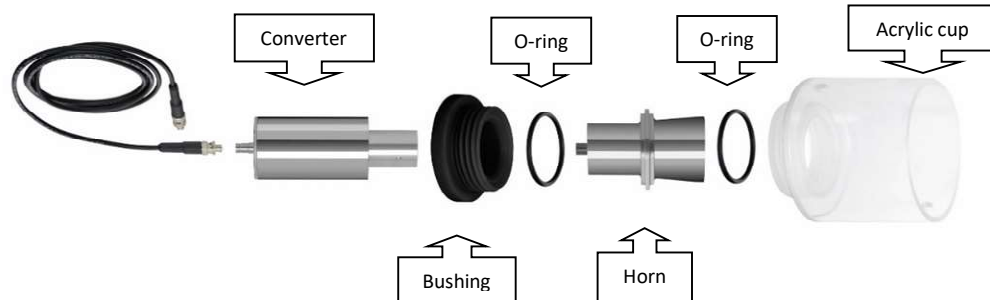
Bridge

Tube Holder

- Hose Barb

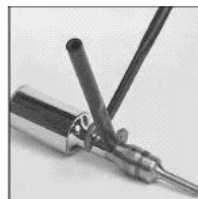
- Plug

The cable and converter are part of the Sonicator system and are NOT included with the purchase of a Cup Horn. The following diagram shows the basic layout of the major components for installation/maintenance.

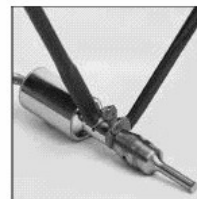


**Installation** If the Cup Horn is purchased separately from the Sonicator, you will need to follow the procedure below:

1. Ensure the power supply is OFF and unplugged. If you have a standard probe on your Sonicator, follow step 2. If not, skip to 4.
2. If a probe is attached, use the spanner wrenches provided with the power supply to remove the probe from the Converter.



REMOVAL



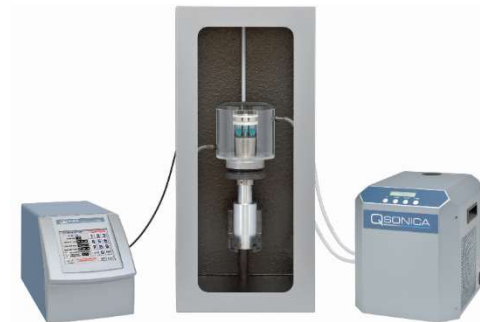
TIGHTENING

3. Place the Cup Horn on the benchtop with the threaded stud facing upwards. Slowly attach the Converter. The threaded stud should easily join the Cup Horn to the Converter. Tighten the assembly using the wrench set.



4. The Cup Horn is supplied with both Plugs and Hose Barb fittings (which are compatible with  $\frac{1}{4}$ " inner diameter [ID] tubing). The Hose Barb fittings must be used if cold water will be recirculated through the Cup Horn to maintain sample temperature. Otherwise, Plug fittings can be used to hold water in the reservoir. Use Teflon tape on all fittings to ensure a water tight seal. It is not necessary to screw the fittings all the way into the Cup Horn.
5. Check for leaks: Fill the Cup Horn with distilled (DI) water to just below the hole of the lower outlet port to ensure that the O-rings, cup and nut are assembled properly and are not leaking water. Drain all the water from the cup reservoir after this step. Caution: do not allow water to drip into the Converter. This can damage its electrical components.
6. Slide the converter mount all the way down to properly support the weight of the filled Cup Horn during sonication. Mount the Converter-Cup Horn assembly in the sound enclosure (part #432B2). Note: *The Cup Horn can generate extremely loud noise levels. Qsonica recommends using the Sound Enclosure accessory to reduce the sound to safe/comfortable working levels.*

The converter cable should be inserted into a side port of the enclosure and connected to the Converter.



7. If temperature monitoring is required (only compatible with Q700 Sonicators), attach the Temperature Monitoring Thermocouple (part #4103) to the Sonicator generator. Insert the Thermocouple through the a side port of the enclosure and place the end of the thermocouple wire into the water.

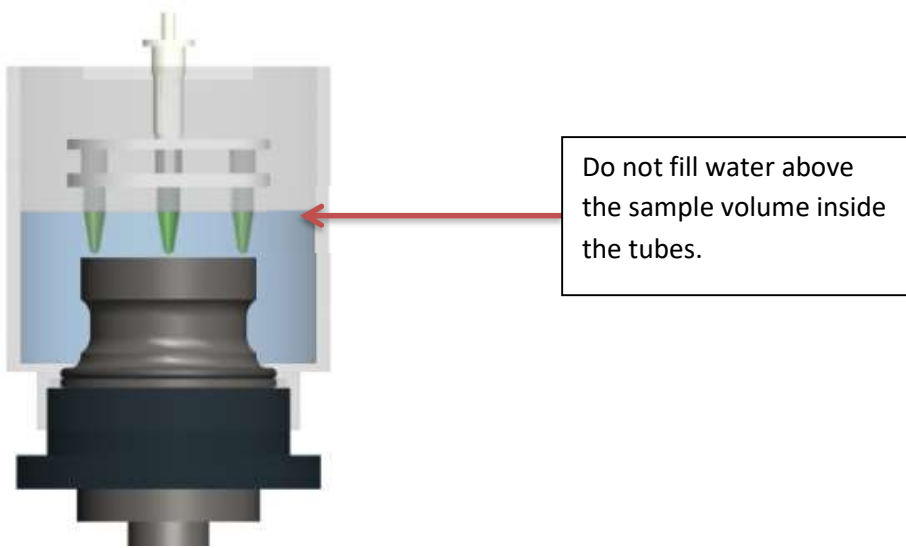
### Setup – Without a Chiller

During operation of the Cup Horn, the ultrasonic energy will cause the water temperature to rise. The Cup Horn can be used in 2 ways: with or without the recirculation of water to cool samples.

1. **Without Chiller** - For brief processing times when chilling the water is not necessary.
  - a. Install Plug fittings (see page 3) into both ports on the cup. If available, wrap threading with teflon tape to ensure a leak proof seal.
  - b. Prepare chilled DI water to fill the Cup Horn.
  - c. Place the Tube Holder and attached sample tube rack with samples into the cup.
  - d. Add water to the cup until the water level is equal to the sample liquid level inside of the sample tube (see the picture below). Once the appropriate water level is determined, use laboratory tape to mark the water fill level on the outside of the cup. *The water level must remain constant to prevent variability when processing samples.*
  - e. If sonication is brief, the heat generated in the cup will not affect the sample. If the water temperature rises during sonication, pour it out (or use a pipette) and refill the cup with cold water to the tape. *Note: To further reduce temperature, crushed ice can be used to help cool the water in the reservoir. Water level must remain constant to prevent variability when processing samples. Be aware that melting ice can change the water level. In addition, ice should only be placed near the edge of the cup. If ice gets under the tubes, it blocks the transmission of ultrasonic energy.*

### Water Level

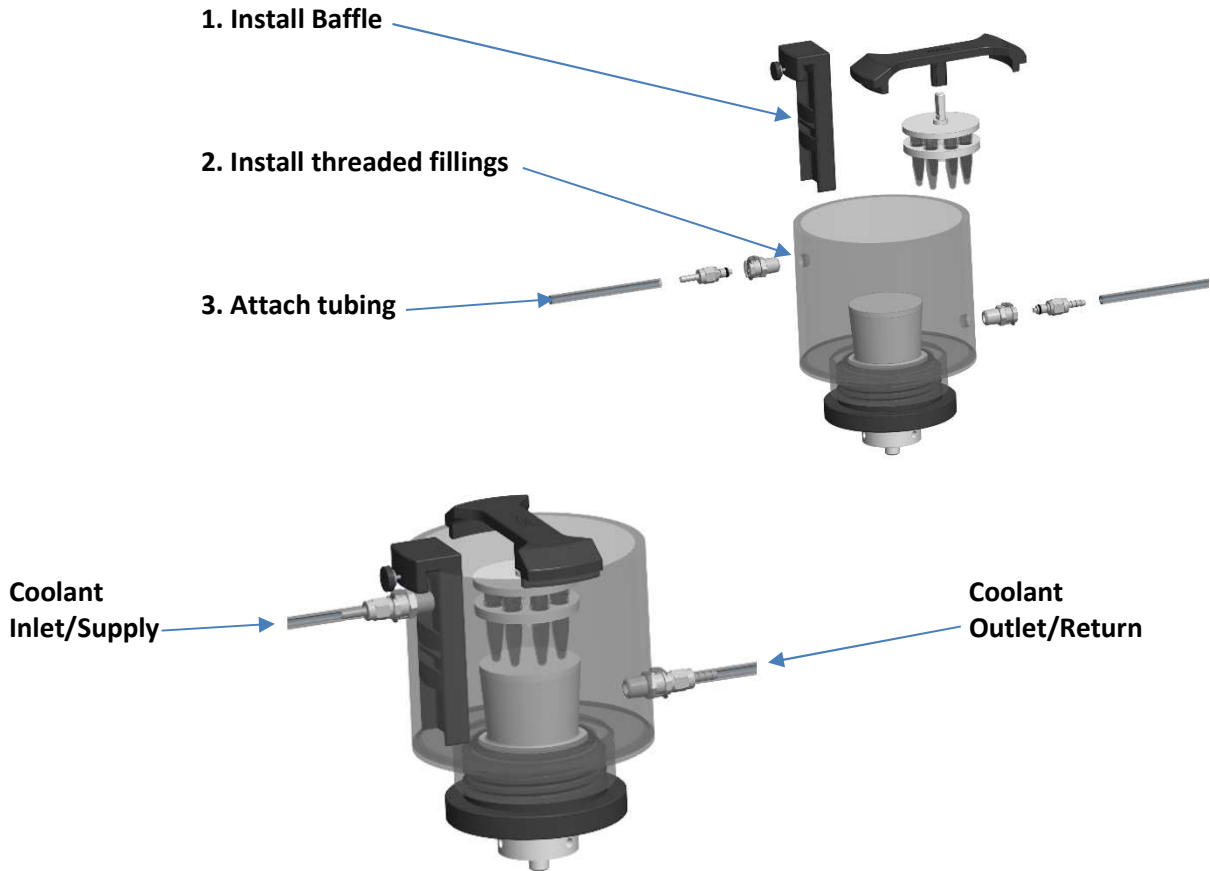
For optimal performance, the water level inside any type of Cup Horn must be equal to the liquid level inside the sample tubes.



**Note:** DI water is recommended.

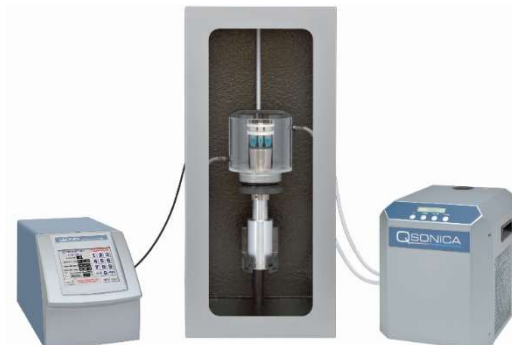
**Setup – with a Chiller**

2. **Recirculating Chiller (#4905)** – is the recommended method to cool the Cup Horn. See the parts associated with the cup horn/chiller set up:

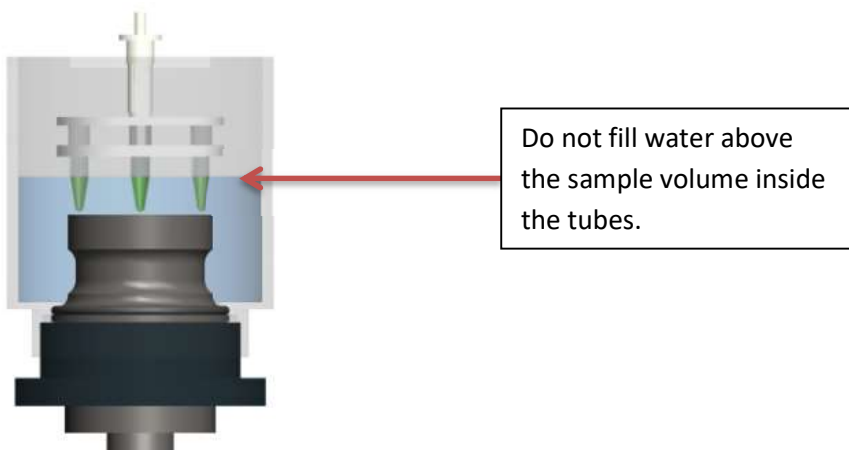


The tubing and connector set (#4911) is required to properly attach the chiller to the Cup Horn. The tubing is labeled to ensure the top fitting is connected to the chiller supply and the lower fitting on the cup is connected to the chiller return.

When using the Sound Enclosure, there are capped ports on either side. Remove the caps and pass the tubing through each port before attaching the connectors.



- a. Pour water directly into the cup to fill the system. Switch on the Chiller. Water will fill the tubing and filter on the back of the Chiller causing the water level to drop. Add water as needed. See image below for recommended water level.



- b. Allow the Chiller to recirculate water through the Cup Horn until a desired temperature is reached before starting sonication. To reduce set up time, store 2L of chilled DI water in the refrigerator for setup.
- c. Note: if your samples are temperature sensitive, it is important to monitor the water temperature in the cup reservoir. Do not rely solely on the temperature reading on the Chiller. The LED display on the Chiller shows the temperature of the water inside the Chiller. The temperature in the Cup Horn during sonication may be a 1-2 degrees higher.



### Choosing Sample Tubes and Racks

It is important to choose the best type of sample tube for your specific application. Smaller tubes (such as 0.5ml PCR tubes) generally work best. When using 1.5ml tubes, Qsonica recommends polystyrene because they work much more efficiently than standard polypropylene tubes. *We do not recommend using standard polypropylene tubes for lysing yeast, shearing chromatin or other difficult to process samples.*

For most applications, processing samples over 1ml is not recommended. When used with the appropriate tubes, all racks hold tubes approximately 4mm above the ultrasonic horn.

Note that specific types of tubes are recommended for each rack. The following tube racks are available:



**#440 – 8 tube holder (Included with Cup Horn)**

Made for use with Evergreen Scientific polystyrene 1.5ml tubes with caps.  
[www.evergreensci.com](http://www.evergreensci.com); Part # 214-3721-010 AND 300-2911-020  
 Must purchase both tubes and caps.  
*Recommended when using 1.5ml tubes.*



**#451 – 8 Tube holder (Included with Cup Horn)**

Made for use with standard 1.5ml Polypropylene tubes (Eppendorf, etc.)  
*Note: Polypropylene tubes do not transmit energy as well as polystyrene.  
 -This is the least efficient option-*



**#449 – 12 tube holder**

Made for use with Brandtech 0.5ml PCR sample tubes  
[www.brandtech.com](http://www.brandtech.com) Part #781310

Thin walled PCR tubes work well for most applications. Best option!



**#445 – 24 tube holder**

Made for use with Brandtech 0.2ml PCR sample tubes  
[www.brandtech.com](http://www.brandtech.com) Part #781305 (thin wall)

Thin walled PCR tubes work well for most applications.

**Note:** Use smaller tubes for smaller volumes. For example: 0.1ml samples fit best in 0.2 or 0.5ml tubes.

**Other Sample Tube/Vessel Information**

Standard 15 and 50ml tube holders are available as well as custom sizes:



**15ml tube holder**



**50ml tube holder**

Most laboratory grade plastic or borosilicate glass vessels that will fit into the cup are suitable for sonication. Polystyrene and polycarbonate are better transmitters of acoustic energy and are recommended. Glassware must be free of scratches or it may crack. Metal sample vessels can be used as well.

Contact us if you have questions about alternative vessel sizes inside the Cup Horn.

## **Operation**

### **Key points:**

- Keeping a constant water level is important for consistent results.
- 500ul tubes work most efficiently and are recommended.
- If you must use 1.5ml tubes, polystyrene is best.
- A Chiller is recommended instead of ice to cool the system.
- Keep cold DI water in a refrigerator/cold room if not using a Chiller.
- Use the pulse mode to allow samples to cool, if necessary.
- The longer the pulse's off duration, the cooler the system will remain.
- If you must use ice, do not allow it to flow under the sample tubes.
- Only add ice to the periphery of the cup to prevent it from obstructing the ultrasonic energy.
- Appropriate amplitude and time settings MUST be determined by empirical testing

Once your samples are in the Cup Horn and the Cup Horn is filled with water to the appropriate level, operate/program the Sonicator as described in the Sonicator manual.

## **Optimization**

Before processing any samples - it is important to run the system and observe the sonication field with water only. Do not put samples inside the Cup Horn. Observe the tiny bubbles traveling through the liquid, which is visual evidence of the ultrasonic energy. The swirling action above the horn may resemble a miniature tornado. As you increase amplitude, the intensity of the sonication increases throughout the water column.

Cup Horns produce a lower intensity than direct sonication methods (standard probes and microtips). Direct sonication settings from a probe system are not transferable to a Cup Horns. Although sonication protocols will be application dependent, many Cup Horn protocols require higher amplitude settings (i.e. 50% or higher) and longer duration times compared to direct sonication.

## **Optimization and Settings**

In order to optimize a sonication protocol you must first minimize sample and setup variability. Begin with one sample volume (i.e. 300ul) and one sample concentration. Load sample tubes in rack and set water level properly.

### **Pulse Mode**

In order to control temperature, program the Sonicator to **pulse ON/OFF** as needed. For example, 20 seconds ON and 20 seconds OFF. Monitor the water temperature and adjust the ON and OFF durations to control temperature. Note that pulse off time can be minimized or eliminated when using a Chiller.

### **Time Course Trial**

A time course trial is required to determine correct amplitude and time settings for each application. Load the tube rack with an appropriate amount of tubes. Select an amplitude setting to start the trial.

Easily processed samples may work well using low amplitude, such as 30%. More difficult applications, such as chromatin shearing, will require higher settings. 50% amplitude is the recommended starting point.

### **Example trial:**

Pulse mode – 20 seconds ON and 20 seconds OFF

50% amplitude

Run samples for 2, 5, 10, 15 and 20 minutes.

(These are just examples and you can develop your own trial)

Some applications may process in a few minutes but difficult sample types may take much more time. If samples are not processed effectively after approximately 20 minutes, increase amplitude and run new trial.

### **Results**

Examine and compare the results to determine processing efficiency at each intensity and time combination. If necessary, complete additional rounds of testing to determine an appropriate sonication time to achieve desired results. Remember to minimize any changes to sample variables (i.e. concentration, volume, etc.) while testing.

**Note:** Changes to sample preparation will have an effect on the required amplitude setting and processing time.

Below are variables that should remain constant (as much as possible) during testing:

- Changes in total sample volume
- Sample concentration
- Temperature
- Cell line
- Cell origin (tissue or culture)
- Type of sample tube used
- Crosslinking fixation time (if you are working with CHIP samples)
- Temperature of water reservoir

## Maintenance

The Cup Horn must be tightened properly to the Converter. Normal use of the Cup Horn can loosen it over time due to ultrasonic vibration. Maintenance schedules will depend on frequency of use. Generally, the Cup Horn should be inspected monthly and cleaned and re-tightened as needed.

The Cup Horn should be disassembled and cleaned periodically with mild detergent. The cup is made of acrylic, so solvents cannot be used within the cup or for cleaning purposes.

**The Cup Horn should not be stored overnight with water inside the reservoir. Keeping the unit dry will inhibit the growth of mold.**

Drain the system (Cup Horn reservoir, tubing and Chiller) at the end of each day. This will prevent the water in the system from becoming stagnant and growing bacteria/mold.

## Periodic Cleaning Procedure

1. Removing the Cup Horn from the Converter may be required every few months if the system is used often. When cleaning the Cup Horn always remove it from the Converter.
  - a. Drain all water from the cup reservoir.
  - b. If Hose Barb fittings and tubing were used to re-circulate water through the reservoir, ensure that they are drained of water and the tubing detached from the cup.
  - c. Unscrew the clear plastic cup from the black nut and set aside.
  - d. Use the pin spanner wrenches provided with the Sonicator system to loosen the cup horn from the Converter as show in the figure below.
  - e. Once the Cup Horn is loose, it can be unscrewed by hand from the Converter. *Be sure to place the Converter on a surface that will not allow it to roll off a bench or table.*



2. Clean all parts with a mild detergent and warm water. The titanium Cup Horn can be autoclaved if necessary. Do not clean plastic parts with solvents or abrasives (may cause the acrylic to crack).

3. Clean the threaded mating surfaces on the horn stud and Converter with a cotton swab and alcohol.
4. Allow parts to dry. Do not allow any liquid to drip into the Converter opening when cleaning the threading. *All surfaces must be dry before assembly and tightening.*
5. Inspect all parts of the Sonicator system and Cup Horn for damage before reassembly. Pay particular attention and check if:
  - a. the threading on the Cup Horn stud and Converter are scratched
  - b. the O-ring is dried out or cracked
  - c. the Cup Horn/fittings leak water into the converter,
  - d. the water tubing is dirty or cracked (only applicable if recirculating water to cool system)
6. Reattach and tighten the Cup Horn assembly as directed in the installation instructions.

Note: Do not attempt to refinish or smooth the horn if it shows erosion after extended use. Wear on the face of the horn is expected over time. Ultrasonic horns are considered consumable parts that will require replacement. The usable life of a Cup Horn is dependent on the intensity setting and the frequency of use. Most customers can expect many years of use before replacement is needed.

**If contamination does occur please take one or more of the following steps to remedy the issue:**

1. Flush the system with fresh water and drain. Repeat.
2. Treat water with Clear Bath® or a similar product to inhibit mold growth.
3. Drain the cup, remove from the Sound Enclosure, and disconnect from the Converter.
4. Remove the cup from the horn and wash with a mild detergent. Rinse and allow parts to dry completely.
5. Reassemble horn and then tighten it to the Converter with the wrench set provided. (see the section below on Cup Horn assembly for assistance).
6. Inspect tubing and replace when necessary.

**As needed:**

1. Horn-Converter connection must be properly tightened. If not, you may see an overload error message, a fluctuation in wattage reading, or a change in the noise level. Please refer to instructions in the Cup Horn assembly section below. Note: A loose horn may damage the generator circuitry, Converter, and/or horn.
2. Check horn for signs of wear with normal usage. Using a severely worn horn can damage internal generator components. Contact Qsonica if the horn's surface shows signs of pitting or excessive wear.
3. Replace tubing as needed.

**Chiller:**

Refer to the Chiller manual for additional maintenance information for this device or the filter that accompanies it.

## **Troubleshooting**

Sonicators are designed to provide you with years of safe and dependable service. However, component failure or improper usage can cause suboptimal equipment performance, overloads, or permanent damage. The most probable causes for malfunction are listed below and should be investigated.

- The horn is not tightened properly with the wrenches provided. Remove and re-tighten.
- A connector or cable is damaged.
- The unit is plugged into an electrical outlet that provides a different voltage from that required. See *Electrical Requirements*.
- The horn is worn past its useful life.
- A fuse(s) has failed.

### **OVERLOAD CONDITION**

If the Sonicator system stops working and an OVERLOAD indication is displayed on the screen, check for possible causes as outlined above. Press the **OFF** key to switch the unit off, and the **ON** key to switch the unit back on to restart the equipment.

***If the problem persists, contact us through the website for repair assistance:***

<https://www.sonicator.com/pages/troubleshooting-repair>