

# Microplate Horn Instruction Manual



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#### **Overview**

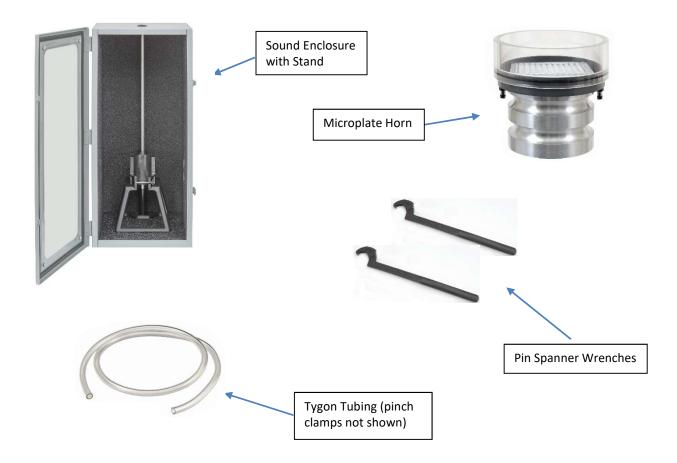
Microplate Horns (MPH) offer indirect sonication and function as high intensity ultrasonic water baths. Multiple samples can be processed in microplates or individual tubes, eliminating aerosols and cross contamination. MPH's are ideal for sterile or pathogenic sample processing and high throughput ultrasonic sample processing.

The ultrasonic horn has a plastic collar mounted to its surface on O-rings. This collar creates a reservoir that must be filled with DI water. A microplate or sample tubes are placed within the reservoir and held above the surface of the horn. The horn creates ultrasonic energy which is transmitted through the water and into each sample.

Cavitation is produced in the water, processing the samples within the sample tubes or microplate. The MPH generates a significant amount of heat and the temperature should be controlled to prevent the samples overheating. Coolant ports are located on each side of the reservoir for circulation of cold water. Qsonica offers #4905 Recirculating Chiller for use with the MPH. Pulsed sonication along with the use of a chiller is highly recommended to adequately control sample temperature.

A sound enclosure is included with the purchase of a microplate horn package. In addition to reducing sonication noise to safe levels, it securely supports the MPH in the proper position. The sound enclosure features ports on either side to allow coolant tubing to pass from the MPH to the chiller.

#### **Parts**







Quantity	Part #	Description
1	432B2MPX	Sound Enclosure
1	853-00074	Silicone O-ring (around the horn – AS568A-248)
1	853-00079	Buna-N O-ring size #255 (around the grey collar)
1	630-0431MP4	Microplate Horn
1	630-0636	Grey Collar
2	859-00156	Hose barb fittings 1/8" NPT / 3/16" ID tube barb
1	M-U8005A431MPD	Clear Deep Well Collar (removeable)
1	529-0063	Baffle for water direction in Microplate horn
1	816-00149	Tygon Tubing
2	868-00714	Pinch Clamps
2	4015	Pin Spanner wrenches
		Converter and cables are included with the purchase of the Sonicator system.



#### **Installation**

Ensure that the ultrasonic processor is OFF and unplugged. Place the power supply on the benchtop and the sound enclosure next to it. The Microplate Horn is shipped attached to the #CL333 ultrasonic converter. See installation of the converter and horn below.

Open the Sound Enclosure and position the Stand inside as shown below. The converter holder has a thumbscrew on the right side. Loosen the screw to position the holder onto the stand as shown in Figure A. Place the converter and Microplate Horn into the converter holder as shown in Figure B.

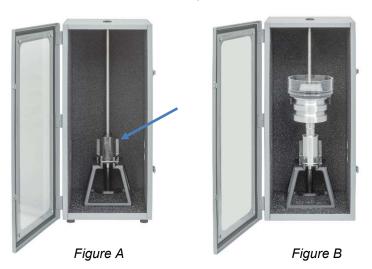




Figure C

After installing the converter and horn, lock the thumbscrew to the support rod. Then lock the horizontal locking pin in the front of the holder. See Figure C.

#### **Installation Without Chiller**

2 pieces of tubing are included. Slide the pinch clamps on and attach to the hose barbs as shown here:





# Chiller Installation Option (recommended setup)

Two pieces of tubing are included with the #4916 tubing set. Feed the coolant tubing through the holes on each side of the sound enclosure.

Note the MPH has a baffle at the surface shown on the left side of this photo highlighted by the arrow. The baffle directs the water for proper flow inside the horn. This is the Inlet side of the horn and the open hole on the opposite side is the Outlet. Match the labels on the chiller tubing to ensure water flows out of the chiller and into the baffle side.

Attach the power cord to the back of the power supply and plug into an appropriate outlet. Attach the converter cable to the back of the power supply and bottom of the converter. The converter cable will go through either port on the side of the sound enclosure.





Check to ensure the O-rings and collars are seated level on the horn. The collars should not be on an angle. Check for leaks when you fill the water reservoir for the first time. Be careful not to allow water to drip into the Converter.



#### Sample tube/vessel Information

The Microplate horn can be used in a variety of configurations in order to process a variety of microplate styles and other sample vessels (i.e. microtubes, PCR tubes, etc.).

Tubes or microplates must not rest on the surface of the ultrasonic horn. Use a Qsonica tube holder when processing individual tubes. Custom tube holders are available.

#### **Microplates**

Open skirted PCR plates work best and are recommended because they allow water to flow freely under and surround each of the 96 individual wells. Half-skirted plates also work well. Flat bottom plates are not recommended. Here are 2 examples of recommended types of microplates:

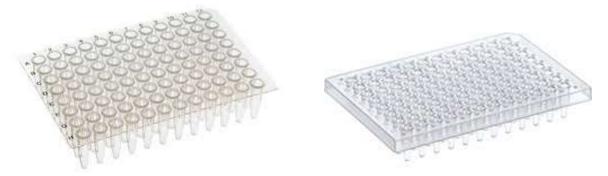


Plate material is also a major factor. Thin wall or polystyrene plates transfer ultrasonic energy most efficiently. Polypropylene or thicker plates absorb much of the energy and result in poor outcomes.

Deep well plates tend to perform less efficiently because they are made with thick plastic material that prevents transmission of ultrasonic energy to the sample.

Plate selection depends on the application. Easy to process samples may work well in any type of plate. But difficult to process samples must be done in our recommended plate format. Contact Qsonica to discuss microplate selection.

Note: Microplate sealing tapes or sealing mats should always be used to prevent sample contamination.

Microplates can be held properly within the Horn by using the #447 plate positioner.







# 1.5ml Tubes

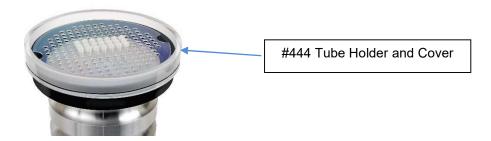
1.5ml tubes can be processed in the MPH. Use #4268 Tube Holder as shown below:



# **PCR Tubes**

Thin walled 300ul PCR tubes are recommended for use with the part #444 sample tube holder and cover.

Note: The tall, clear collar must be removed before using this holder.









#### **Operation**

#### **Recirculating Chiller Mode** - (Recommended method)

- 1. Set up the system as shown in the instruction manual.
- Fill a container with 500ml of cold DI water. Slowly pour about 200ml into the horn and then turn on the chiller. The chiller will pull the water into the tubing and filter. Keep adding water until the filter in the back of the chiller is full and the horn has the appropriately 1cm of water inside.
- Place the microplate or tube rack with samples into the reservoir. Look closely to ensure that air is not trapped under the microplate as it will prevent ultrasonic energy from reaching sample wells and cause inconsistent results.



- 4. Adjust the water level until it just covers the sample liquid level within your microplate or tube. If the #447 plate positioner is not used, adjust the water level to allow the microplate to float a few millimeters above the surface of the horn. Once the appropriate water level is determined you may wish to use laboratory tape to mark this level on the outside of the reservoir. Use the same level each time to ensure consistent results.
- 5. Allow the Chiller to recirculate the water through microplate horn until a desired temperature is reached before beginning sonication. To reduce set up time store/use chilled DI water for startup.
- 6. Note the LED display on the chiller shows the temperature of the water as it leaves the chiller. The actual temperature in the microplate horn during sonication may be 1-2 degrees warmer. The temperature will fluctuate during sonication.
- 7. Use pulse mode as required to maintain your desired temperature if needed. The converter should remain near room temperature at all times. The reservoir water temperature should not exceed 60°C (140°F). If either of these temperature limits are reached, shut the system off and allow to cool.
- 8. Appropriate amplitude and time settings must be determined by empirical testing.

#### Static Reservoir (For brief processing times and when temperature is not an issue)

- 1. A single piece of tubing is attached to each hose barb on the underside of the horn.
- 2. Have a source of chilled DI water to fill the microplate horn.
- 3. Place the microplate or tube rack with samples into the reservoir. Look closely to ensure that air is not trapped under the microplate as it will prevent ultrasonic energy from reaching sample wells and cause inconsistent results.
- 4. Adjust the water level until it just covers the sample liquid level within your microplate or tube. If the #447 plate positioner is not used, adjust the water level to allow the microplate to float a few millimeters above the surface of the horn. Once the appropriate water level is determined you may wish to use laboratory tape to mark this level on the outside of the reservoir. Use the same waterlevel each time to ensure consistent results.
- 5. If using ice the ice should only be placed at the periphery of the microplate/sample tubes and never under the sample vessel which would block the transmission of ultrasonic energy. Note ice is



not recommended but we know many customers do try it.

- 6. Pulse mode is required to help keep samples from overheating. The converter should remain near room temperature at all times. The reservior water temperature should not exceed 60°C (140°F). If either of these temperature limits are reached, shut the system off and allow to cool.
- 7. Appropriate amplitude and time settings must be determined by empirical testing.

#### **Optimization**

The first time you use the Microplate Horn, it is important to run the system and observe the sonication field without samples (water only). Notice the tiny bubbles traveling through the liquid, as this is visual evidence of the ultrasonic energy. As you increase amplitude the intensity of the sonication increases and travels through the water column.

Microplate horns produce a lower achievable intensity than direct sonication methods (standard probes and microtips). The microplate horn may require longer processing times when compared to a probe system. Sonication settings used with a probe are not transferrable to a microplate horn system.

#### **Optimizing Sonication Protocols**

In order to optimize a sonication protocol you must first minimize both sample and set up variables. Begin with one sample volume and one sample concentration. Load samples and set water level properly. Below is an example of an optimization experiment. Intensity setting and total sonication time can be adjusted depending on how difficult the specific sample is to process. Note this is just an example.

- 10% amplitude for 1, 5 and 10 minutes sonication ON time
- 25% amplitude for 1, 5 and 10 minutes sonication ON time
- 50% amplitude for 1, 5 and 10 minutes sonication ON time

In order to control temperature, program the sonicator to pulse On/Off as needed. Examine and compare the results to determine lysis or shearing efficiency at each intensity and time setting. An example pulse mode may be 10 seconds on/20 seconds off. Pulse mode can be adjusted as needed.

Complete additional rounds of testing if necessary. Minimize sample variability while testing.

**Note:** Once an amplitude setting that best processes the sample is determined, adjust the total sonication time until a desired result is achieved. Once an optimal sonication intensity setting is determined for a specific sample, changes to a sample variable (such as volume or concentration) may require adjustment to the total sonication time to achieve the same results.

Examples of sample and preparation variables that will have an effect on sonication protocols and can possibly require adjustments can include (but are not limited to):

- Changes in sample volume
- Sample concentration
- Temperature
- Cell line
- Cell origin (tissue or culture)

Examples of sonication set up variables that will have an effect on sonication protocols and will possibly require adjustments can include (but are not limited to):

- Type of sample tube used
- Temperature of water reservoir
- Distance samples tubes are from the ultrasonic horn



#### **Microplate Horn Maintenance**

The microplate horn should be drained each night. Keeping the unit dry will inhibit the growth of mold and prevent the aluminum horn from oxidizing. Wipe the surface dry each night. The tubing and chiller should be drained each week.

Oxidation of the metal surface is normal (white cloudy precipitate forms). Simply rinse the horn surface and wipe any precipitate away with a clean soft cloth. The microplate horn surface will naturally change color and will wear (surface will become rough and pitted) over time with use. Like all sonicator horns, it will require replacement after the surface is pitted and performance is no longer satisfactory. Horn lifespan depends on usage and amplitude settings so a timeframe cannot be estimated.

It is recommended that the microplate horn be disassembled and cleaned periodically with a mild detergent. The clear collar is made of acrylic, therefore solvents cannot be used within the reservoir or for cleaning purposes.

The microplate horn should always be tightened properly to the converter. With normal use the microplate horn can loosen over time due to ultrasonic vibration. The microplate horn should be inspected monthly, cleaned and re-tightened as needed.

# **Daily Cleaning**

Drain water from the system. This will prevent the water from becoming stagnant and growing bacteria/mold. Wipe the surface of the horn dry each day.

#### **Draining Instructions**

Place a 500-1,000ml beaker or container on the benchtop. The water from the MPH will drain into this vessel.

# **Draining the MPH Without a Chiller:**

- 1. Place both of the open ends of the two sections of tubing into the vessel.
- 2. Open the white plastic pinch clamps on the tubing and the water will drain.
- 3. Wipe the surface of the horn dry.



Pinch Clamps

#### **Draining with a Chiller:**

1. Disconnect the 2 pieces of clear tubing from the chiller with the white quick disconnect fittings. These are labled Coolant supply and Coolant return. Leave the other ends connected to the MPH.





- 2. Locate the blue drain fitting as shown to the right. Place the end of one section of tubing inside the beaker.
- 3. Attach the blue drain fitting over the white fitting and water will immediately drain out of the tubing and into your vessel.
- 4. Once the first section of tubing has drained, repeat the procedure with the other section of tubing.
- 5. Wipe the surface of the horn dry.

# Drain fitting

#### **Draining of the Chiller:**

- The Coolant Supply tubing should be disconnected from the chiller.
- 2. Connect the Drain Tube with blue fitting to the Coolant Supply port. Insert the open end of the tube into your vessel.
- 3. Turn on the chiller and it will pump out the water until it runs dry. Shut off as soon as the water stops flowing.
- 4. Locate the filter on the back of the chiller. Using the filter wrench, unscrew the filter housing and pour out the water. Allow the filter and housing to air dry when not in use.

# Drain Tube

#### If contamination does occur follow these steps:

- 1. Disconnect the tubing from the horn. Horn can only be washed with water and mild detergent. Disconnect the filter from the chiller. Wash filter housing with soap and water and put aside for next step.
- 2. Flush the chiller and tubing only with a 10% bleach, 90% water mix. Run the chiller with a beaker full of this mixture for approximately 10 minutes. Drain and rinse with clean water for several minutes. Discard tubing and connectors and replace with new ones.
- 3. Remove the collar from the horn and wash with a mild detergent. Rinse and allow parts to dry completely. Reassemble horn and then tighten it to the converter with the wrench set provided. (See the section on microplate horn assembly in the installation instructions for assistance).

#### As needed basis:

- 1. Horn/converter must be properly tightened. If not you may see an overload error message, large fluctuations in wattage readings or a change in the noise level. Please refer to instructions in the microplate horn assembly section to retighten the converter/horn assembly.
- 2. Check horn for signs of wear with normal usage. Using a severely worn horn can damage internal generator components. Contact us if the horn's surface shows signs of excessive wear.
- 3. Replace tubing and connectors each quarter or as needed.
- 4. Replace chiller filter each quarter or as needed. Do not leave the filter housing full of water when not in use for extended periods.



After draining remove the converter from the MPH using the spanner wrench set. Ensure the converter is set aside and not exposed to water. The converter is not covered by the warranty if it is determine that there was water inside.



Remove the microplate horn from the converter

- 1. The MPH can be cleaned with a mild detergent and warm water. The aluminum horn alone can be autoclaved if necessary. Do not clean with solvents or abrasives.
- 2. Clean the threaded mating surfaces on the horn stud and converter with a cotton swab and alcohol.
- 3. Allow parts to dry. Do not allow any liquid to drip into the convertor opening when cleaning the threading. All surfaces must be dry before assembly and tightening.
- 4. Inspect all parts of the Sonicator system and microplate horn for damage before re-assembling the microplate horn. Pay particular attention to the threading on the microplate horn stud and converter. The o-ring should not be dried out or cracked, the horn/fittings should not leak water into the converter, if using tubing to re-circulate water it must be replaced if dirty or cracked.
- 5. Reattach and tighten the microplate horn assembly as directed in the installation instructions.

Note: The horn is a tuned resonant body of aluminum alloy. Do not attempt to resurface the horn if it shows erosion after extended use. Wear on the face of the horn is normal over time and is expected.



# **Troubleshooting**

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 or stop working all together. The most probable causes for malfunction are listed below and should be investigated.

- The system has overheated. Investigate water temperature and discuss with Qsonica.
- The horn is not tightened properly with the wrenches provided.
- A connector or cable is damaged.
- The unit was plugged into an electrical outlet that provides a different voltage from that required. See *Electrical Requirements* in Sonicator Manual.
- The Horn being operated is worn past its useful life.
- A fuse(s) has failed.

#### **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 **OFF** key to switch the unit off, and the **ON** key to switch the unit back on to restart the equipment.

Please contact Qsonica with any questions.