

ChIP/DNA Shearing

Sonicators are commonly used for ChIP Assay and DNA shearing. The most common method is to insert a microtip probe directly into a sample. At Qsonica, we call this direct probe sonication. Energy is transmitted from the probe directly into the sample and the sample is processed very quickly.

For some applications, direct sonication is not preferred. For example, if you are processing very small samples (500ul or smaller) a probe may cause foaming or aerosolization. In addition, if you are processing many samples, using a probe can be very tedious and time consuming.



Indirect sonication is a great option for processing very small samples as well as multiple samples. Cup Horns offer indirect sonication. Individual sample tubes are arranged in a floating rack and placed inside the cup, which is filled with water. Ultrasonic energy is transmitted from the titanium horn within the cup, through the water and into the sample tubes. Cup Horns have become very popular for ChIP/DNA shearing applications. Issues associated with probe sonication (foaming, aerosolization, sample loss, over-heating and the tedium of doing one sample at a time) are eliminated.

Microtip probes deliver very high intensity sonication. When using a Cup Horn, the ultrasonic energy is transmitted through your microtube into your sample. Consequently the sonication intensity of a Cup Horn is lower than that of a microtip. Due to this difference in intensity, sonication parameters (time and amplitude settings) will vary when comparing the two methods. Cup Horns will effectively process a sample but require a longer processing time than a microtip.



Some sample types will shear very quickly and easily. Other samples are more difficult and may require higher intensity settings and longer processing times. All Sonicator systems require optimization by the user. There are several variables that must be addressed in order to obtain your desired results when using a Cup Horn. The following information will help to explain these variables, minimize their effect and improve your results.

Cup Horn Set Up Variables

- 1. Amplitude/Intensity setting:** The higher the amplitude setting the more energy that will be delivered to your sample, and the quicker your sample will be processed. However, higher amplitude settings cause the water in the cup horn to heat up more quickly.
- 2. Control the temperature of the water in the cup horn:** During operation the cup horn will generate heat. The water must be cooled. We recommend using a recirculating chiller bath and a dual head peristaltic pump. It is important to keep the water level constant so the tubes do not move and create another variable that can affect your results. Ice chips can also be used but will not keep temperature levels as consistent. If ice is used, be sure it is added on top or on the sides of the tubes. Ice below the tubes may have a negative effect on your results.
- 3. The bottom of your sample tubes may touch the surface of the ultrasonic horn:** Touching the horn will provide the best transmission of ultrasonic energy into your sample tube. Insert the rack of tubes into the cup. Fill the cup with as much water as possible but do not let the tubes float. If the tubes float off the horn, remove some water. Depending on what the tubes are composed of, some tubes may be brittle or prone to cracking when they are exposed to long periods of ultrasonic energy. We recommend running a test of your sample tubes with the recommended water level, with only buffer in the tubes to see if the tube material is affected.

- 4. Sample tube composition:** Polystyrene or Polycarbonate allow for the best transmission of ultrasonic energy. Polypropylene sample tubes are more commonly available but will absorb some of the energy before it reaches the sample. More difficult to shear samples will benefit from using Polystyrene or Polycarbonate tubes.
- 5. Sonication duration:** Every sample is different. Some will shear in as little as a few minutes and some may take an hour of processing time. This is why it's important to use a full-featured Sonicator with programmability and temperature monitoring. Programming your sonicator to pulse on and off will help to control temperature. Total sonication time will vary with different cell lines and sample concentrations that are harder to shear than others.
- 6. Sample tube rotation:** Some customers with more difficult to shear samples have found it may be beneficial to rotate their tubes. Since processing time will vary, you will need to set your own rotation schedule. For example, if you are processing for 15 seconds on, with a rest between sonication, turn the samples a quarter turn each rest period.
- 7. Degas liquid in reservoir:** Degassed liquids will transmit energy more efficiently. Simply sonicating the water in the cup horn for several minutes will degas the liquid.

Variability of Samples

It is extremely important to note that there are assay condition variables that will affect sonication results. While optimizing your sonication procedure you must keep these details constant.

1. Concentration Formalin/Formaldehyde used (1% or less is recommended; some have used as little as 0.1%)
2. Sample Volume
3. Sample Concentration
4. Cell line/type
5. Cross-linking fixation time (10 minutes or less have been recommended)
6. Keep Antibody lot constant (different lots will require sonication protocol variations)

Note Your Sonicator and Sample Parameters

Keep a record of the following parameters during sonication for reproducibility:

1. Energy delivered (Joules reading)
2. Amplitude (intensity) setting
3. Total sonication time
4. Pulse rate (On and Off time intervals)
5. Water temperature; also record flow rate of recirculating liquid
6. Sample tube composition
7. Biological assay sample conditions (sample volume, sample concentration, cell line, fixation time, etc...)
8. Any other sample conditions you feel affect the results of sonication