

# Talboys Cryogenic Homogenizing System Manual

## Quick Start

The following are a list of key points to use the Cryogenic Homogenizing System safely and effectively.

- **DO NOT OVERCHARGE HOMOGENIZER. DO NOT EXCEED 12 HOURS OF CHARGING TIME.**
- Liquid nitrogen is needed to cool the Cryogenic Homogenizer's mortar and pestle, and to pre-chill the sample to cryogenic temperatures. Liquid nitrogen is -196°C and can cause burns. Wear safety gloves, safety glasses, and a laboratory coat when handling liquid nitrogen. Review organizational safety procedures for using liquid nitrogen.
- Grinding the pestle against the mortar **without** a sample causes accelerated wear to these items. The dust generated from this "**dry grinding**" is a respiratory hazard. **DO NOT DRY GRIND.** Always grind with a sample in the mortar.
- The porcelain/zirconium mortar and pestle are resistant to abrasion, but can be cracked by impact. Avoid dropping or impacting the mortar and pestle.
- Samples of 100 mg or less are more effectively ground than larger samples. For larger samples, it may be necessary to mix the sample with the tip of a pointed spatula (chilled first) several times during the grinding.
- To transfer the ground sample to a tube (e.g., 15 ml centrifuge tube), ensure that the powdered sample is not compacted in the bottom of the

mortar (loosen with a chilled spatula if necessary), invert the tube and place over the opening in the mortar, and invert tube and mortar and tap the powdered sample into the tube.

- Decontaminate the mortar and pestle following processing. The mortar and pestle can be autoclaved. Once clean, the mortar and pestle can be further decontaminated by dry heating to 200°C for 2 hours or by rinsing with decontamination solution.

## **Introduction**

The Cryogenic Homogenizing System is a miniaturized mortar and pestle system that combines the effectiveness of manual grinding with the convenience of a handheld homogenizer. The Cryogenic Homogenizing System is designed for processing multiple small samples, i.e., less than 0.5 grams and most effective at 100 mg or less, by reducing the size of the mortar and motorizing the pestle. The small size of the mortar also makes transferring the pulverized sample to a tube very efficient.

Like the traditional application of cryogenically grinding with a mortar and pestle, the Cryogenic Homogenizing System makes use of liquid nitrogen to chill the mortar, pestle, and sample prior to grinding. The cryogenic cooler that accompanies the complete system contains a rack that accommodates up to six mortars, six pestles, and a reservoir for liquid nitrogen which maintains cryogenic temperatures. The complete system includes a small mortar composed of a porcelain/zirconium composite, a porcelain/zirconium pestle adapted with a motor compatible shaft, a motorized unit, and a cryogenic cooler with rack.

## **Safety and Precautions**

Liquid nitrogen is an essential component of cryogenic grinding. Liquid nitrogen has a temperature of  $-196^{\circ}\text{C}$ , it has the potential to cause severe burns of the skin and eyes. Materials and items that come into contact with liquid nitrogen or liquid nitrogen vapor are also extremely cold. Consequently, **DO NOT** let liquid nitrogen or chilled items come into contact with hands, skin, or eyes. Wear protective gloves, laboratory coat, and safety glasses when handling liquid nitrogen. Review safety rules and procedures established by your lab/organization for handling liquid nitrogen.

Dry grinding the mortar and pestle, i.e., grinding without a sample in the mortar, will cause unnecessary wear on the components and generate porcelain dust. Porcelain dust can be a respiratory health hazard, therefore, **DO NOT** grind without a sample. If dust is generated, simply rinse the mortar and pestle with water to remove.

The Cryogenic Homogenizer is designed to be powered by a low speed motorized unit. This motorized unit has a speed of 300 rpm and is considerably slower than handheld homogenizers. This slow speed is important as it prevents sample splattering and the generation of airborne particles from the sample. However, as a precaution, a dust mask should be worn if the samples contain bio-hazardous materials and grinding should be performed in a biological safety hood. Furthermore, following homogenization of bio-hazardous materials, work surfaces should be decontaminated with 70% isopropanol or similar disinfectant, and mortar and pestles should be autoclaved.

Prior to using the Cryogenic Homogenizer, the mortar and pestle can be sterilized by autoclaving or with dry heat. Normal autoclave cycles are suitable for the mortar and pestle, i.e., 121°C for 15 min. Dry heat sterilization can be done at 200°C for 2 hours. The pestles should not be heated above this temperature. Allow mortars and pestles to cool before handling. Allow the mortar and pestles to thoroughly dry before exposing to liquid nitrogen.

### **Instructions for Use**

The Cryogenic Homogenizing System is most effective on small samples of 100 mg or less, though samples up to 500 mg can be processed. The following is a general procedure for grinding samples.

**DO NOT OVERCHARGE HOMOGENIZER. DO NOT EXCEED 12 HOURS OF CHARGING TIME.**

1. The Cryogenic Homogenizer 's mortars and pestles can be prepared by washing with a biologically compatible detergent and then by rinsing thoroughly with water. Allow the mortar and pestle to completely dry before using. If a sterile grinding is needed then the mortar and pestle can be autoclaved at 121°C for 15 min. Allow to dry before use. If a nuclease-free and nucleic acid-free grind is needed, then the mortar and pestle can be treated with a commercial decontamination solution and/or dry heat sterilized at 200°C for 2 hours. Do not heat the mortar and pestle above 200°C.

2. Place the Cryogenic Homogenizer's mortar and pestle into the Cryogenic Cooler, tray, or pan (at least 2 inches deep) that can withstand liquid nitrogen. If a pan is used, place the pan on top of an insulating layer, such as the lid to a Styrofoam lab cooler. This will protect the bench surface from the extreme cold of the liquid nitrogen. Prior to adding liquid nitrogen to the pan/tray or Cryogenic Cooler (i.e., liquid nitrogen reservoir), wear appropriate protective clothing (glasses, gloves, lab coat) and prepare the work area so to prevent accidental spills.
3. Harvesting and preparing tissues for cryogenic grinding is a vital step in obtaining good results. Many bio-molecules, such as mRNA, is quickly degraded in cells, thus rapid freezing after harvested is necessary. Perhaps the most efficient method for freezing tissue is to rapidly harvest and drop the tissue into liquid nitrogen. However, liquid nitrogen can be a source of contamination and the potential effect on the experiment should be considered. Alternatively, a metal plate (e.g., sheet of 1/8" thick aluminum or stainless steel) can be chilled in liquid nitrogen vapors and used as a "cold skillet" to quickly freeze tissue. The metal acts as a cold sink and pulls heat from the sample in seconds. Once frozen, most tissues can be stored before processing. Samples that will be used for RNA isolation should be stored at temperatures below the glass transition point of water, or  $-120^{\circ}\text{C}$ . At this cryogenic temperature, all biological activity is stopped. At ultralow temperatures, e.g.,  $-80^{\circ}\text{C}$ , RNA will still degrade, albeit more slowly than in a standard refrigerator or freezer.

Tissue that will be harvested and stored in liquid nitrogen freezers for repeated analysis should be quickly cut into thin strips or tiny cubes (5 mm<sup>3</sup>) as long as positional integrity of the tissue is not important. Samples frozen in this manner are much easier to process than tissues stored in large solid chunks. Whether plant or animal, large cryogenically stored tissues are very difficult to dissect for processing without warming them up.

4. Pour liquid nitrogen into the reservoir. With the Cryogenic Cooler, the liquid nitrogen will be adsorbed by an adsorbent underneath the wire mesh. Add sufficient liquid nitrogen so that a shallow pool of liquid nitrogen approximately ½" or 1-2 cm forms above the mesh. If using a pan or tray, add liquid nitrogen to a depth of about 1 inch. Initially the liquid nitrogen will boil vigorously as the reservoir cools, however after several minutes the boiling will subside. Place the mortar and pestle into the pool of liquid nitrogen. Allow the mortar and pestle to chill with in the Cryogenic Cooler, close the lid to slow the evaporation of the liquid nitrogen. With a pan or tray, a Styrofoam lid or box can be placed over the pan if a sufficiently large box is available. NOTE: An alternative to the above directions is to place the mortar and pestle in the reservoir and then fill with liquid nitrogen. This is a faster method, but it does not prevent the mortar and pestle from becoming contaminated from the liquid nitrogen. Liquid nitrogen is not sterile and can be highly contaminated, especially if it is retrieved from a tank where cell lines in plastic cryogenic vials are

stored submersed. Consequently, mortars and pestles that are directly exposed to the liquid nitrogen can not be considered sterile or nucleic acid and nuclease free. However, depending upon the intended use of the samples ground in the Cryogenic Homogenizer, such minor contamination may be inconsequential, especially if the homogenized sample will be used for enzyme assays.

5. Small samples are most efficiently pulverized with the mortars and pestles. For best results, sample should be smaller than 5 mm<sup>3</sup> which is about 125 mg of tissue. Samples can be pre-frozen (as described above) or frozen upon harvesting by dropping into a pre-chilled mortar (allow several minutes for the sample to come down in temperature). Protect your hands against the cold by wearing gloves. Choose gloves that fit well, allow dexterity, but still prevent cold burns. With the sample in the mortar, fit the hex nut of a chilled pestle into the motorized unit, hold the mortar firmly with a gloved hand, and tap down on the sample with the mortar. Grind by pressing the forward button on the motorized wrench while pressing down firmly on the sample. Grind for a couple of seconds and then reverse direction with the wrenching. Repeat this forward and reverse grinding for a total of 15-20 seconds. Examine the sample. It should appear as a fine powder and at this point can be transferred to a tube. If large particles are still present, repeat the grinding. See step 6 if the sample is large.

6. For larger samples, it may be necessary to mix the ground tissue several times during the processing. To do this, chill a thin metal spatula and use the tip to dislodge any compressed tissue. Continuing grinding with the pestle. Repeat the process until the tissue is finely ground.
7. Once pulverized, the sample can be transferred to a tube (e.g., 15 ml centrifuge tube) for further processing or storage. Using the spatula, loosen any sample that is compacted on the bottom of the mortar. To transfer, invert the tube and place over the opening in the mortar, invert tube and mortar together, and tap the powdered sample into the tube. Check the mortar for residual sample, loosen and tap into the tube as necessary. Typically, recovery of the sample is greater than 95%, much higher than a traditional mortar and pestle that yields less than 70%. Keep the sample cold following the transfer until needed.
8. Following grinding, allow the mortar and pestle to warm up to room temperature before decontaminating and cleaning. Refer to step 1 for cleaning guidelines.

### **Care and Maintenance**

The Cryogenic Homogenizer's mortars and pestles can be routinely re-used if cared for properly. The porcelain/zirconium composite that is used to craft the mortars and pestles is extremely wear resistant, as it is more durable than even the hardest biological samples, such as bone. However, the mortar and pestle will wear significantly when used against each other in the absence of sample, referred to here as **dry grinding**. The mortar and pestle will produce a visible

powder, or dust, when used without sample. This powder is a result of wearing of the mortar and pestle, and dry grinding will reduce the life of these parts. **DO NOT DRY GRIND WITH THE MORTARS AND PESTLES. ALWAYS GRIND WITH A SAMPLE.**

The porcelain/zirconium composite used to form the mortar and pestle is very wear resistant, however it is a ceramic-based item and sensitive to impact. **Avoid dropping or impacting the mortar and pestle on hard surfaces.**

The mortar and pestle can be autoclaved and heat sterilized. **DO NOT USE DRY HEAT ABOVE 200°C.** Allow the mortar and pestle to dry and/or cool before using and exposing to liquid nitrogen.

The mortar and pestle can be washed with detergents, though it is suggested that biologically compatible detergents be used to prevent carryover contamination of biological samples. One good detergent/wetting agent is 1% sodium carbonate solution. It is both effective and inexpensive.