

## CryoGrinder™ System User Guide

### Quick Start

The following is a list of key points for using the CryoGrinder™ safely and effectively.

- Liquid nitrogen is needed to cool the CryoGrinder™ mortar and pestle, and to pre-chill the sample to cryogenic temperatures. **CAUTION:** 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 the CryoGrinder™. 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, invert tube and mortar together, and tap the powdered sample into the tube.
- Decontaminate the mortar and pestle following processing by autoclaving. Once clean, the mortar and pestle can be further decontaminated with dry heat at 200°C for 2 hours or by rinsing with decontamination solution.

The following are a list of key points for using the CryoCooler™ safely and effectively.

- The CryoCooler™ uses liquid nitrogen (LN<sub>2</sub>) to reach and sustain a cryogenic temperature within the cooler (< -130°C). **CAUTION:** Liquid nitrogen has a temperature of -196° C, which can cause severe burns. Therefore it is necessary to wear safety goggles, gloves, and a laboratory coat while working with LN<sub>2</sub>.
- The CryoCooler™ features a pan that is 5.5” x 7” x 5.5”. A newly designed rack with handles was also added to assist with the handling and holding of samples.
- Disinfect and clean the CryoCooler™ prior to and after use, by spraying the surfaces with 70% isopropyl alcohol and then wiping to dry. **DO NOT** clean the surface with a solvent such as acetone, because it will degrade the cooler’s insulation.
- Fill the reservoir with 1 liter of LN<sub>2</sub>, wait 5 minutes then fill with LN<sub>2</sub> until a **thin layer** of LN<sub>2</sub> is visible above the surface of the metal grating.
- **DO NOT** fill the metal reservoir to the top. This can cause an overflow of LN<sub>2</sub> which can become a safety concern may cause damage to the CryoCooler™.
- **DO NOT** pour any liquids in the reservoir other than liquid nitrogen.
- Leaving the lid open for an extended period of time after charging will cause loss of liquid nitrogen vapor and reduce cryogenic working time.

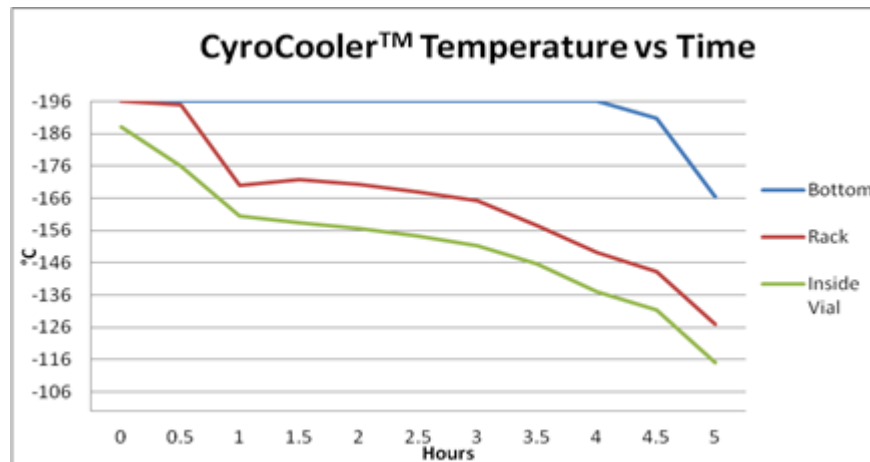
## Introduction

The CryoGrinder™ is a miniaturized mortar and pestle system that combines the effectiveness of manual grinding with the convenience of a handheld homogenizer. The CryoGrinder™ 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 larger mortar and pestle, the CryoGrinder™ makes use of liquid nitrogen to chill the mortar, pestle, and sample prior to grinding. The optional cryogenic cooler contains a rack that accommodates up to twelve mortars and a reservoir for liquid nitrogen which maintains cryogenic temperatures. The complete system includes eight small mortars composed of a porcelain/zirconium composite, 16 porcelain/zirconium pestles (two different sizes) adapted with a motor compatible shaft, a motorized torque wrench, and a cryogenic cooler with racks. The components can be purchased individually, as a kit, or as a system.

Cryogenic sample preparation can be a difficult process because of the challenge of maintaining an optimal cryogenic temperature below  $-130^{\circ}\text{C}$  under standard conditions. Biological molecules such as RNA are susceptible to degradation at room temperature and are best harvested at cryogenic temperatures. Thus, it is important to maintain a temperature well below  $-130^{\circ}\text{C}$  when working with such labile molecules.

The CryoCooler™ was designed as a portable, cryogenic station aimed at increasing working time during sample preparation and collection. Once the chamber is fully charged with liquid nitrogen it will maintain temperatures well below  $-130^{\circ}\text{C}$  for up to 4 hours (see graph below). Consequently, the CryoCooler™ can be used for sample collection outside of the laboratory and for processing samples in the laboratory. The CryoCooler™ is suited for sample transport, preparation, sample collection in operating rooms, and short-term storage. **It is not designed for overnight shipping of cryogenic samples.** When used in conjunction with the CryoGrinder™, a miniaturized mortar and pestle it is effective for disrupting samples of less than 100 mg within a cryogenic environment.



## Safety and Precautions

- Liquid nitrogen (LN<sub>2</sub>) is necessary to charge the CryoCooler™ to the appropriate cryogenic temperature, thus several pre-cautions must be taken to prevent injury.

**CAUTION: Liquid nitrogen has a temperature of -196 °C and will cause severe burns.**

- Any materials and items that come into contact with the LN<sub>2</sub> or its vapors will also become very cold and can cause burns. Therefore, safety goggles, gloves, and a laboratory coat must be worn while working with LN<sub>2</sub>.
- 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 CryoGrinder™ is designed to be powered by a **low speed** torque wrench. This torque wrench has a speed of less than 300 rpm and is considerably slower than handheld homogenizers. This slow speed is important; 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 biohazardous materials and grinding should be performed in a biological safety hood. Furthermore, following homogenization of biohazardous materials, work surfaces should be decontaminated with 70% isopropanol or similar disinfectant, and mortar and pestles should be autoclaved or chemically disinfected.
- Prior to using the CryoGrinder™, 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. Never submerge or expose the torque wrench directly to liquid nitrogen.
- The torque wrench used for powering the pestle is a standard commercial tool that has been tested in this system. Review the manufacturer's documentation for this tool that has been enclosed prior to its use.
- Direct contact of LN<sub>2</sub> with the outside of the container may cause paint to chip if exposed for a period of time. Review the safety rules and procedures established by your lab/ organization for handling liquid nitrogen in addition to the ones provided by this manual.
- Never overfill the CryoCooler™ the maximum capacity of the reservoir is **3 Liters**. The liquid nitrogen should never rise above the tray with metal grating (see diagram).
- **NEVER** clean the CryoCooler™ with acetone. It will degrade the cooler's insulation. **DO NOT** submerge or rinse the cooler with water or any other substance.
- Cleaning the CryoCooler™ can be done by first removing the perforated platform and Liquid Nitrogen Pillow and then disinfecting the chamber by wiping surface with 70% isopropanol.

## Preparation of Sample

Harvesting and preparing tissues for cryogenic grinding is a vital step in obtaining good results. Many biomolecules, such as mRNA, are quickly degraded in cells, thus rapid freezing after harvest 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 of that contamination 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°C. At this cryogenic temperature, biological activity ceases. At ultralow temperatures, e.g., -80°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>) assuming 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 chunks of cryogenically frozen tissues are very difficult to dissect for processing without warming them up.

## Instructions for Use

The CryoGrinder™ is most effective on small samples of 100 mg or less, though samples up to 500 mg can be processed. Following is a general procedure for grinding samples.

- 1. Prepare the CryoGrinder™ with detergent, decontamination solution and/or autoclaving.**
  - Detergent cleaning: Prepare mortars and pestles by washing with a biologically compatible detergent and then by rinsing thoroughly with water. Allow the mortar and pestle to completely dry before using.
  - Autoclaving for sterilization: The mortar and pestle can be autoclaved at 121°C for 15 min. Allow the CryoGrinder™ to dry before use.
  - Nuclease-free/Nucleic acid-free: 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 CryoGrinder™ pestles above 200°C.
- 2. Disinfect with 70% isopropanol prior to use.**
  - Sanitize its surfaces by wiping them with 70% isopropanol. **DO NOT** submerge the cooler or pour any other liquid beside liquid nitrogen into the reservoir.
- 3. Wear safety goggles, gloves, and laboratory coat.**
  - Working with LN<sub>2</sub> can cause burns to skin and clothing.
- 4. Verify the metal tray with grating is secure.**
  - Open the CryoCooler™ lid and ensure the metal tray is in place by tightening the thumb nuts before.
- 5. Charge the pillow with 1 liter of liquid nitrogen.**
  - Pour 1 liter of liquid nitrogen into the reservoir, close the lid, and wait 5 minutes. Take caution when initially charging the cooler. The LN<sub>2</sub> will violently boil until the CryoCooler™ has cooled. **DO NOT** submerge the cooler in liquid nitrogen or place in a cryogenic freezer to pre-chill it.
- 6. Complete the charge to the CryoCooler™ after the initial chilling.**
  - Slowly add additional liquid nitrogen to the reservoir until a thin layer of LN<sub>2</sub> is visible above the tray with metal grating. After the boiling has stopped and the LN<sub>2</sub> levels have dropped, additional LN<sub>2</sub> may be added. **It is important to never exceed a thin layer of liquid nitrogen above the metal grating.** Overfilling the CryoCooler™ may damage the paint on the outside of the cooler and internal insulation. Once charged, the CryoCooler™ is ready to use.
- 7. Pre-chill mortars and pestles.**
  - Place the two aluminum Z-brackets on top of the tray with the corresponding sides facing up. The Z-brackets allow the motors and pestles to rest at an angle and still allow for the lid to close. Place mortars and pestles into the CryoCooler™ and pre-chill for 10-20 minutes.
  - **NOTE**: Leaving the lid ajar for extend periods of time will cause the cooler to warm quicker than normal.
- 8. Set a timer for temporary storage or sample processing.**
  - Temporary Storage: The samples can be stored in the cooler for **up to 4 hours** with the lid closed.
  - Sample Processing: Leaving the lid ajar for extend periods of time while processing samples will cause the cooler to warm quicker than normal, and thus an optimal cryogenic temperature of below -130°C may be maintained for **up to 3 hours** with the lid open.
- 9. Monitor the CryoCooler™ temperature (optional).**
  - A digital thermometer can be used to monitor the cooler's internal temperature. The CryoCooler™ can be recharged if the temperature rises above -130°C by following steps 4-6 above.

**10. Sample size should be less than 125 mg and pre-frozen if possible.**

- Small samples are most efficiently ground in the CryoGrinder™. 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 or frozen upon harvesting by dropping into a pre-chilled mortar (allow several minutes for the sample to come down in temperature).
- **NOTE:** An alternative to the above directions is to place the CryoGrinder™ mortar and pestles on the Z brackets in the reservoir and then fill with liquid nitrogen. This method is quicker, but it does not prevent the mortar and pestle from becoming contaminated by the liquid nitrogen. **CAUTION:** 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 submerged. Consequently, mortars and pestles that are directly exposed to the liquid nitrogen should not be considered sterile or nucleic acid and nuclease free. However, depending upon the intended use of the samples ground in the CryoGrinder™, such minor contamination may be inconsequential, especially if the homogenized sample will be used for enzyme assays.

**11. Hold the mortar with a gloved hand for best homogenization results.**

- The mortar needs to be held for operation, so protect hands by wearing gloves. Choose gloves that fit well, allow dexterity, and still prevent cold burns. With the sample in the mortar, fit the hex nut of a chilled pestle into the torque wrench, hold the mortar firmly with a gloved hand, and tap down on the sample with the mortar.

**12. Grind the sample in a forward in reverse direction for 15-20 seconds.**

- Grind by pressing the clockwise button on the motorized wrench while pressing down firmly on the sample. Grind for a couple of seconds and then grind counter clockwise for the same duration. 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.

**13. Use a chilled spatula to mix larger samples for thorough homogenization.**

- 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.

**14. Transfer sample into tube with spatula.**

- Once pulverized, the sample can be transferred to a tube (e.g., 15 ml centrifuge tube) for downstream processing or storage. Using a chilled 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.

**15. Warm mortars and pestles to room temperature prior to cleaning.**

- 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

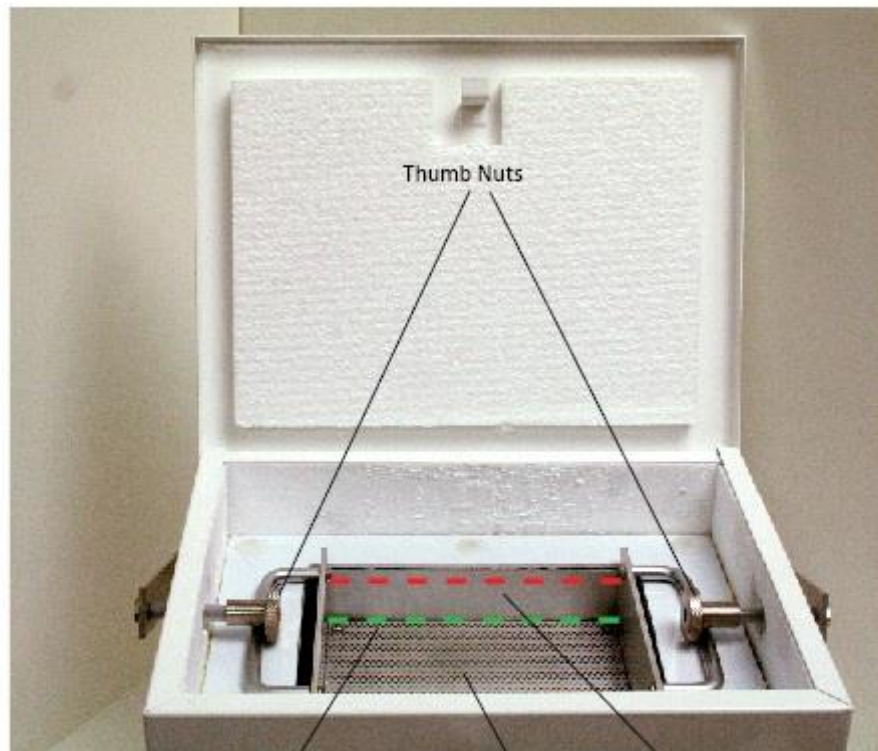
- The CryoGrinder™ mortar and pestle can be routinely re-used if cared for properly. The porcelain/zirconium composite that is used to craft the CryoGrinder™ is extremely wear resistant as it is more durable than even the hardest biological samples, such as bone. However, the mortar and pestle will wear 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 the CryoGrinder™. **DO NOT DRY GRIND WITH THE CRYOGRINDER™. 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.
- The CryoCooler™ can be re-used on a regular basis if it is cared for properly. However, certain substances such as acetone can degrade the insulation.
- Wipe the CryoCooler™ surface clean with 70% isopropanol.
- **DO NOT** fill the metal reservoir to the top. This can cause an overflow of LN<sub>2</sub> which can become a safety concern and may cause damage to the CryoCooler™
- **DO NOT** allow LN<sub>2</sub> to come into contact with the outer box.
- Routinely check the CryoCooler™ reservoir when not in use for the white cellulose absorbent powder residue. If large amounts of white powder are found in the reservoir or on the surface of the perforated metal grate, replace the pillow.

## Related Products

CryoGrinder™ Kit (Mortar and Pestle Kit)	Product No. CG 08-01
CryoGrinder Set (Mortar and Pestle Set)	Product No. CG 08-02
Liquid Nitrogen Absorbent Pillow (Replacement Pillow)	Product No. CG 08-08
CryoStorage™ Box (81 Vial Capacity)	Product No. CRY 012-01
CryoVials™ Sterile (1.8 ml)	Product No. CRY 300-02

## CRYOCOOLER™ OVERFILL WARNING

Please be aware that overfilling of the CryoCooler™ may cause damage to the unit, the paint and be a safety concern. Take note of the maximum fill line. Liquid nitrogen should never rise above the metal grating on the tray. Ensure that the thumb nuts are tight securing the metal tray down before filling. If accidental overfill occurs, do not touch the sides or bottom of the cooler. Allow excess liquid nitrogen to evaporate and the CryoCooler™ to warm before moving.



Thumb Nuts

Tray with  
metal grating

**MAXIMUM FILL LINE**

Never exceed a thin layer of liquid nitrogen above the metal grating.

**OVERFILL WARNING**