

CryoGrinder™ User's 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.

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 six mortars 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 torque wrench, and a cryogenic cooler with rack. The components can be purchased individually, as a kit, or as a system.

Safety and Precautions

- Liquid nitrogen (LN₂) 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 LN₂ 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 LN₂.
- 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.

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 mm³) assuming positional integrity of the tissue is not important. Sample 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. Pre-chill mortars and pestles (optional).**
 - Place mortars and pestles into the CryoCooler™ or pan (at least 2 inches deep) that can withstand liquid nitrogen and pre-chill for 10-20 minutes. If a pan or tray is used, place the reservoir on top of an insulating layer, such as the lid to a Styrofoam lab cooler, which will protect the bench surface from the extreme cold of the liquid nitrogen.
 - Wear safety goggles, gloves, and laboratory coat if working with LN₂. Working with LN₂ can cause burns to skin and clothing.

- 3. 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 mm³ 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.
- 4. 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 if homogenizing at cryogenic temperatures. 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.
- 5. 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.
- 6. 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.
- 7. 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.
- 8. 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.

Related Products

CryoGrinder™ Kit (Mortar and Pestle Kit)	Product No. CG 08-01
CryoGrinder Set (Mortar and Pestle Set)	Product No. CG 08-02
CryoStorage Box (81 Vial Capacity)	Product No. CRY 012-01
CryoVials Sterile (1.8 ml)	Product No. CRY 300-02
CryoCooler™	Product No. CG 08-07
Liquid Nitrogen Absorbent Pillow (Replacement Pillow)	Product No. CG 08-08
CryoGrinder System™	Product No. CG 08-10