

**Cole-Parmer**<sup>®</sup>  
sampleprep

# CG-400/CG-450 Freezer/Mill<sup>®</sup>

Cryogenic Grinder Designed for Milling Tough and Temperature Sensitive Samples

## Operation Manual

For 115V (04500-40, 04500-41) and 230V (04577-92, 04577-93)



For Product Information



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SPEX SamplePrep is now part of Cole-Parmer®.

The Cole-Parmer® CG-400/CG-450 Freezer/Mill was formerly known as SPEX 6875/6875A Freezer/Mill.

Over the years, we've acquired many high-quality and reputable brands. After many years of continual growth, we realize our brands are all as brilliant as each other. Rather than have a portfolio of complementary brands, we felt consolidating them under one world-class brand name enabled us to offer a single and significant brand experience. Through one brand we can speak in one voice through our team of experts who provide support in product selection, usage and troubleshooting to empower laboratories to run efficiently throughout the world.

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## **1.0 INTRODUCTION**

Cole-Parmer Freezer/Mills are laboratory mills that cool unconventional materials to cryogenic temperatures and pulverize them to a powder form without thermal degradation. Our proven technology uses a dual electromagnetic grinding chamber that rapidly drives a steel impactor back and forth against the two end plugs of the sample vial. Since the vial is securely closed the integrity of its contents is maintained, hazardous or critical samples are easily controlled, and cross-sample contamination is eliminated. The sample chemical composition is preserved as a result of the vials being immersed in liquid nitrogen at cryogenic temperatures throughout the grinding process. These unique aspects have made Cole-Parmer Freezer/Mills the most effective cryogenic mills in the world. They are the “mills of first choice” for many abnormal materials, or samples whose composition or structure cannot be ground using conventional grinding methods.

***NOTE: Please do not operate the CG-400/CG-450 Freezer/Mill® until you have read these instructions and are familiar with its controls and operation. The Freezer/Mill is different in its principles and operation from any other laboratory mill.***

***OPERATING CONDITIONS:*** Do not set up the CG-400/CG450 Freezer/Mill® in an insulated or confined space. The Freezer/Mill should be run on an open countertop, in ambient air, with recommended maximum relative room humidity 70% from 5°C - 31°C (40°F - 88°F). If the entire mill is chilled during operation, components such as the gas cylinders, controller, and display screen can fail. This damage is not covered by the Freezer/Mill warranty. During operation, humidity in the air will condense on parts of the unit. For this reason, it is important not to place any moisture-sensitive equipment near the Freezer/Mill.

***VERY IMPORTANT:*** Liquid nitrogen not only makes samples brittle through severe chilling, making them “grindable,” but also cools the magnetic coil which powers the CG-400/CG-450 Freezer/Mill. If the mill is operated without liquid nitrogen for a period of about one minute, the coil will become very hot and may sustain permanent damage. The CG-400/CG-450 Freezer/Mill has a liquid nitrogen sensor that should shut down the mill when the liquid nitrogen gets too low to cool the coil. Nevertheless, the nitrogen level should be visually checked during extended runs as well as topped off before every run (CG-400 model only). The Freezer/Mill warranty does not cover damage to the coil caused by operating the mill with little or no liquid nitrogen.

***SAFETY:*** Liquid nitrogen (LN) can be hazardous. Its boiling point is -195.8°C (-320.4°F). When working with liquid nitrogen directly or indirectly, the LN Tank valve or hose, or chilled Freezer/Mill components, cryogenic gloves must be worn to protect hands. A face Shield is also recommended to protect eyes from possible splashing. Be careful not to splash liquid nitrogen onto clothes or unprotected skin. Wear the proper protection equipment (PPE). Additional information on LN safety can be found at <http://engineering.dartmouth.edu/microengineering/ln2.html>.

## 2.0 CG-400/CG-450 FREEZER/MILL SPECIFICATIONS

<b>Type of Mill:</b>	Cryogenic impact mill
<b>Controller:</b>	Touchscreen
<b>Data Transfer:</b>	Dual USB Port (back of controller)
<b>Grinding Mechanism:</b>	Steel impactor driven by dual electromagnets
<b>Coolant:</b>	Liquid nitrogen (LN)
<b>Dimensions:</b>	20½ in. (52 cm) x 21½ in. (55 cm) x 18 in. (46 cm)
<b>Weight:</b>	(empty, without vial or coolant) approx. 44 lbs. (20 Kg)
<b>Grinding Vials:</b>	One Large Vial (6801), one Mid-Size Vial (6871, 6881, 6885), one to four Small Vials (6751, 6761, 6771, 6781S) or one Microvial set (6757).

**Typical Vial Capacity:** ***Small Vials:*** actual volume with impactor, approx. 25 ml. Typical sample weights: 2 grams for biological samples, 1 gram for polymers.  
***6757 Microvial Set:*** 100 - 500 mg per individual 6757V Microvial, depending on sample.  
***Mid-Size Vials:*** up to 5 times the sample capacity of Small Vials.  
***Large Vials:*** Approximately twice the sample capacity of Mid-Size Vials, or up to 10 times that of Small Vials.  
 Actual performance of any vial/sample combination depends on sample properties, cooling time, grinding time, desired outcome, etc.  
 Effective capacity for a given sample is determined by experiment.  
 Overfilling a vial greatly reduces its efficiency.

**Typical Liquid Nitrogen Consumption:** 10-15 liters for initial cool-down and filling of the tub.  
 4-6 liters for each hour of operation. Actual LN consumption can vary.  
 A minimum 200L LN Tank is recommended.

**Electrical Specifications:** CE Approved. Available as 115V/60HZ or 230/50HZ.

**Circuit Breaker:** Same as ON/OFF Switch

**Power Cord:** 115V/60HZ version: 3-prong grounded plug supplied.  
 230V/50HZ version: 2-prong European plug supplied.  
***The operator is responsible for supplying alternate line cord/plug.***

**Safety Features:** Lid Interlock prevents mill from running if lid is not latched shut.  
 Liquid nitrogen sensor shuts down mill if LN level is too low.

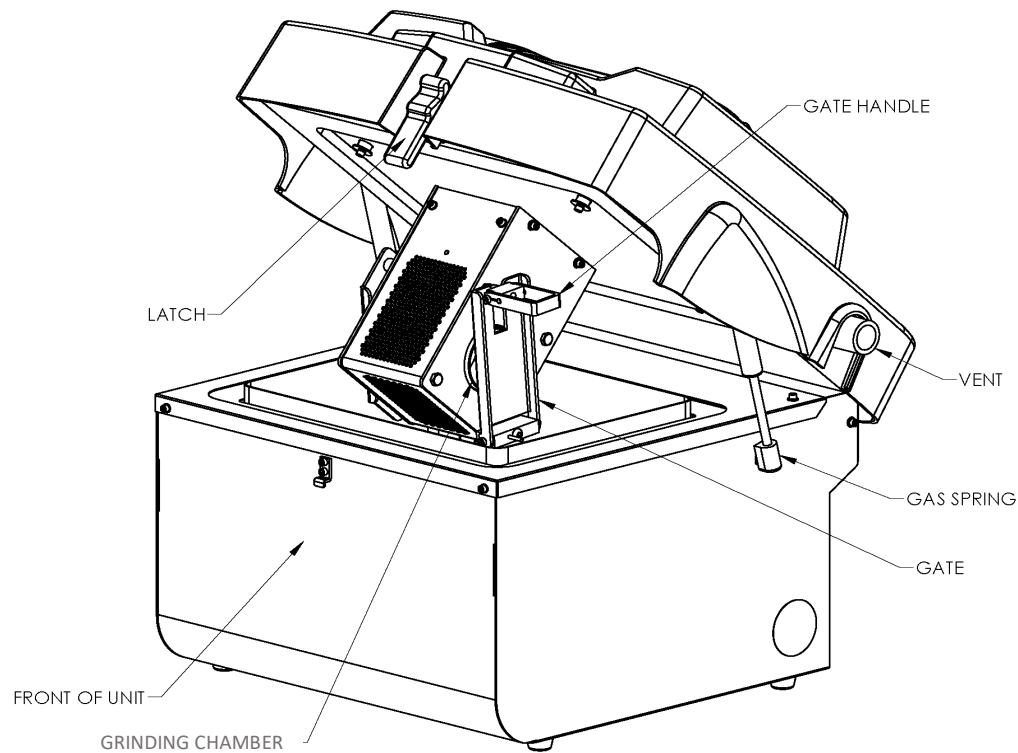
***NOTE:*** *The sensor can shut down the mill during a run, so the liquid nitrogen level should be visually checked during extended runs as well as before every run. The Freezer/Mill warranty does not cover damage to the coil caused by operating the mill with little or no liquid nitrogen.*

### **3.0 UNPACKING**

Inspect the exterior of the packing box and report any visible damage to the carrier. Remove all packing documents from the exterior of the box, and save them for your records. Open the top of the shipping box. Remove the packing material and accessories, and gently remove the CG-400/CG-450 Freezer/Mill. Visually check the mill for any damage that may have occurred during shipping. Unlatch the lid and inspect the interior of the tub, ensuring that it is free of any packing material. Check the packing list to see that there are no parts missing, and inspect the accessories. We recommend storing the packaging materials in the event there is a need to return the unit for warranty service or repairs.

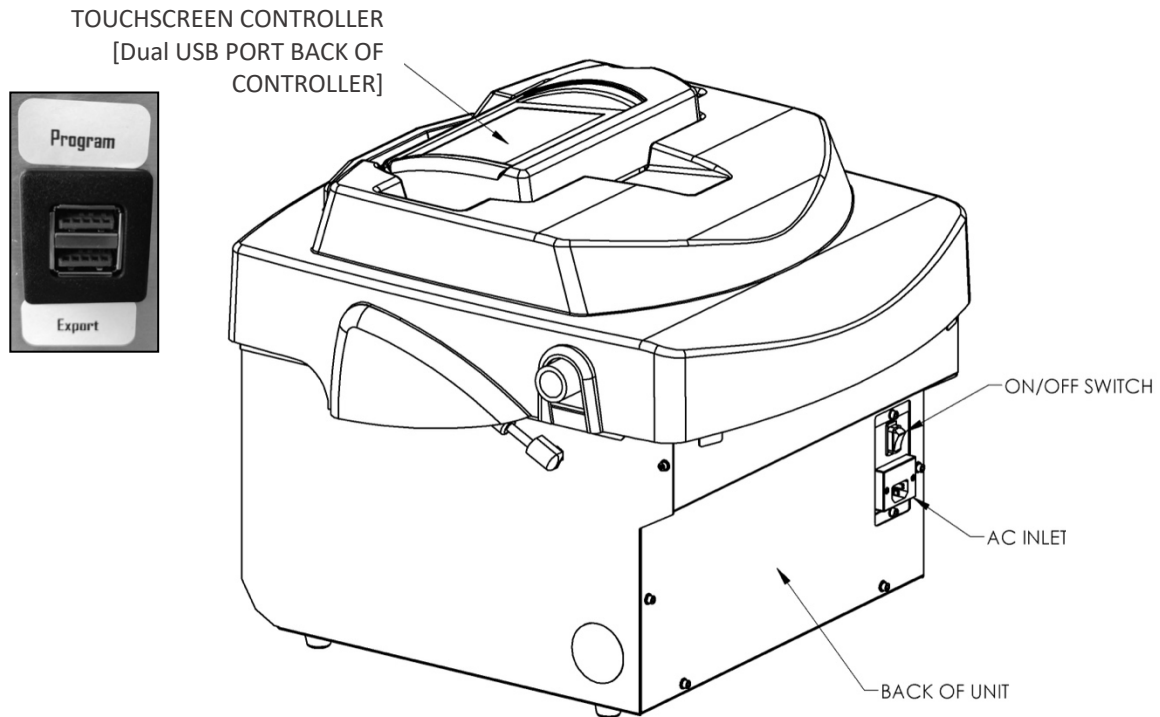
Grinding vials are necessary for processing samples but must be purchased separately. The full range of grinding vials and accessories for the CG-400/CG-450 Freezer/Mill is described in our catalog, the Freezer/Mill Accessory Manual, and at Cole-Parmer.com.

Inspect the accessories. Check the packing list to see that there are no parts missing. If everything seems to be in proper order, store the packaging materials, in case there is a need to return items.



**Figure 1 - CG-400 Freezer/Mill, Front View**

### 3.0 UNPACKING (Cont'd)



**Figure 2 – CG-400 Freezer/Mill, Rear View**

### 4.0 SETTING UP

The CG-400/CG-450 Freezer/Mill weighs 44 pounds (20 Kg) empty. The lid opens from the front and the lid latch is in the center. The AC Input module and the ON/OFF switch (which also serves as a circuit breaker) are on the back of the cabinet. There are gas cylinders on both sides of the cabinet to control the movement of the lid. The controller is affixed to the top of the lid and linked to the unit by an RJ45 Ethernet cable connector. At the back of the controller is a “dual” USB Port to upload and download data, or to upgrade software.

#### **4.1 Electrical Connection**

Plug the electrical cord into the inlet on the back of the Freezer/Mill cabinet, and then into a standard 3-prong grounded electrical outlet. The 230V/50HZ, CG-400/CG-450 Freezer/Mill is supplied with a standard European 2-prong plug with cord. For the 230V/50HZ version, make sure the cord and plug conform to local electrical codes.

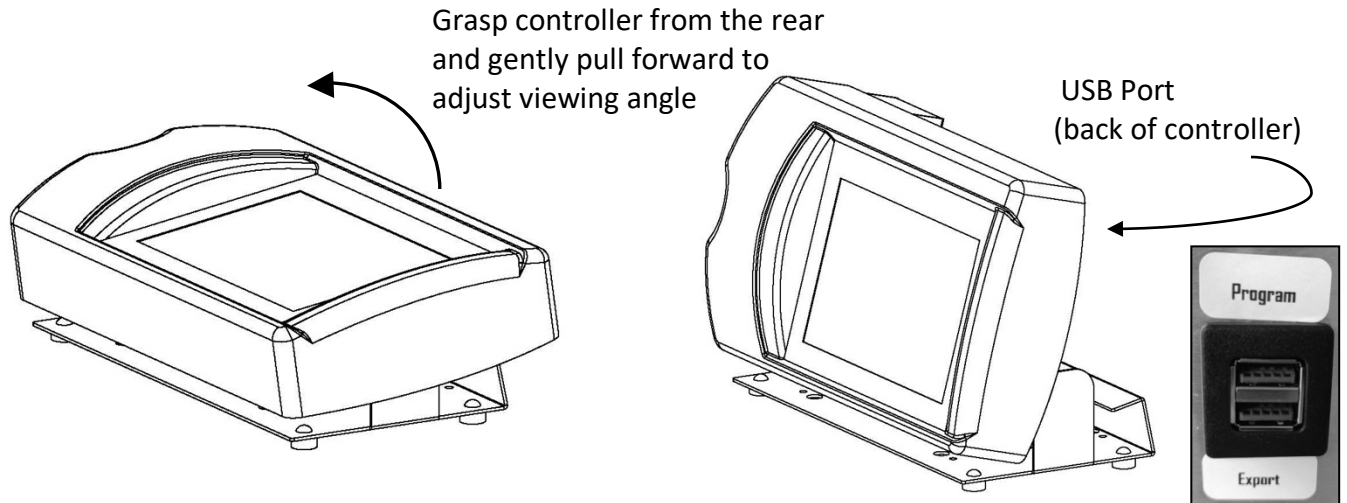
#### **4.2 Power Switch and Controller**

Electric power to the Freezer/Mill is controlled by a rocker switch on the inlet module. It is marked with two numerical symbols (0 for OFF and I for ON). Press the “I” side of the switch to turn the power ON, or press the “0” side of the switch to turn the power OFF. When the power to the mill is turned ON, the controller display will light up.

The controller screen is a touchscreen that can be activated by a fingertip or stylus. Data is transferred to other computer devices via “Export” USB Port, located in back of the controller. The CG-400/CG-450 Freezer/Mill has one grinding chamber, which is programmed on the Control Panel screen (Section 5.2). The programmable parameters include Cycles (number of grinding periods), Precool Time (initial chilling of sample), Run Time (grinding period), Cool Time (time between grinding periods), and Rate (speed of the impactor in cycles per second). The Control Panel also displays a warning if the lid is not fully closed, or if there is not enough liquid nitrogen in the tub.

The viewing angle of the controller on the CG-400/CG-450 Freezer/Mill can be adjusted to minimize glare. To adjust the viewing angle, simply grasp the back of the controller box and gently pull it forward until the desired angle is reached, as shown in Figure 3. If it is necessary to send the unit back to Cole-Parmer or service, be sure to return the controller to its horizontal position before packing the unit for shipping.

#### 4.2 Power Switch and Controller (Cont'd)



**Figure 3** - Adjusting the Viewing Angle of the Controller

#### 4.3 Lid, Coil Assembly, and LN Sensor

The lid of the CG-400/CG-450 Freezer/Mill supports the coil (dual electromagnet assembly) and coil housing. To open the lid, hold the lid down with one hand, and with the other pull the upper end of the latch toward you. The lower end of the latch should disengage from the cabinet. Release the lid and it should be pushed up by the gas cylinders, which also keep the lid raised when open. The lid must be open for insertion and removal of vials.

A vial is inserted into the circular hole on the right side of the chamber housing. The gate keeps the vial or vials in place during grinding, but must be lowered to insert or remove vials. **Do not run the CG-400/CG-450 Freezer/Mill without a loaded vial in the chamber.**

The lid must be shut and latched for the Freezer/Mill to run. Push the lid down with one hand, and hold it down while engaging and closing the latch.

The chamber gate (Figure 1) holds the vial or vials in the chamber during grinding. To lock a vial in place, loosen (unscrew) the chamber gate handle a turn or two, and position it so the handle passes through the slot in the chamber gate when the gate is raised. Tighten the chamber gate handle against the gate, so the gate contacts the vial firmly, but **do not overtighten the gate** as this can crack the plastic center section of the vial. When the chamber gate handle is properly tightened, fold it down to lock the gate during grinding. Always use the proper Adapters for Mid-Size and Small vials (see Section 4.4), and **always wear gloves when touching a chilled vial or any part of the chilled chamber housing!**

### **4.3 Lid, Coil Assembly, and LN Sensor (Cont'd)**

The liquid nitrogen (LN) sensor is the small tube mounted on the left wall of the LN tub. If there is not enough liquid nitrogen in the tub to start the mill when the run button is touched, **LN LEVEL LOW** screen (Section 5.4) will display LN LEVEL LOW. If the LN runs low during a grinding program the same message will appear and the program will stop. To continue running, fill the tub with liquid nitrogen to the mark on the back of the tub. When the run button is pressed, the grinding program will begin where it left off.

### **4.4 Grinding Vial Sizes, Adapters, and Vial Openers**

Large vials (6801, 6871) are run one at a time in the CG-400/CG-450 Freezer/Mill, without any adapter. Large vials are opened using the 6804 Extractor/Vial Opener, which is part of the 6870L Accessory Package or the optional 6808 Large Vial Extractor.

Mid-Size vials (6881, 6883, 6885) are run one at a time, but the 6887 (6888) Mid-Size Adapter must be placed in the chamber first, to center and align any Mid-Size vial. Mid-Size vials can be opened by the 6804 Extractor/Vial Opener, but only by installing the 6884 Mid-Size Vial Adapter for the 6804. The 6884 and 6887 Adapters comprise the 6870M Accessory Package.

Most Small vials (6751, 6761, and 6771) can be run one, two, three, or four at a time in the CG-400/CG-450 Freezer/Mill. However, they must be used in conjunction with the 6807 Multi-Vial Holder. The 6807 Adapter keeps the vials parallel to each other, so each impactor moves back and forth in line with the magnetic field. When running Small vials, make sure the chamber gate contacts the end plug of every vial. If the gate needs adjustment to do this, the bottom or pivot end of the gate can be moved toward or away from the chamber housing by turning the gate clockwise or counterclockwise on its mounting bolt.

## **5.0 TOUCHSCREEN DISPLAY**

The CG-400/CG-450 Freezer/Mill is programmed and operated through a series of touchscreen displays. Transition between screens, and all programming and operating commands, are done by touching the screen with a fingertip or stylus. ***Do not use a sharp point as this can damage or deface the screen.***

### **5.1 Logo Screen**

When the power is switched ON at the back of the mill, "FreezerMill" appears during start-up as the software loads. Then the screen will switch to the **Home Screen**, as shown in Figure 4. From the Home screen the Control Panel, Saved Protocols, Run History, and Settings can be accessed by touching the buttons displayed.

## 5.1 Logo Screen (Cont'd)

The Home Screen Icon can be found on the following screens (e.g. Control Panel, Saved Protocols, Run History, Settings) positioned at the top right of the screen. Touching the Home Icon allows the users to return to Home Screen.

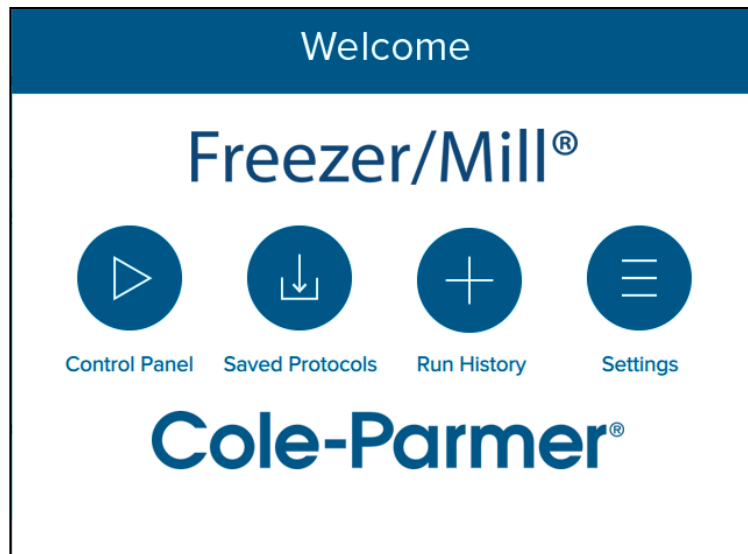


Figure 4 – Home Screen

## 5.2 Control Panel

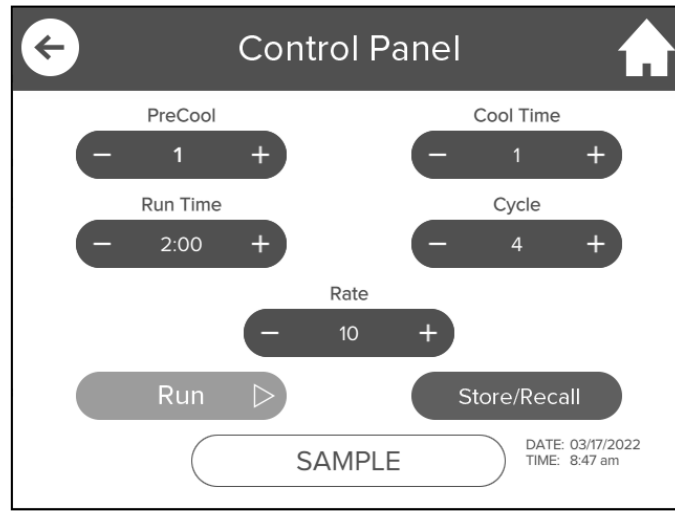
***NOTE:*** To keep a run from being interrupted by the liquid nitrogen (LN) sensor, the LN level should be visually checked from time to time during extended runs, as well as before every run.

The Control Panel displays the programmed run parameters, as shown in Figure 5. Changes to the run settings, are made from this screen. The CG-400/CG-450 Freezer/Mill is **Only** equipped with one Grinding Chamber.

To recall stored run protocols, touch the store/recall button located at the bottom right of the screen. (See section 5.4 for more information on Saved Protocols)

--To return to the Home Screen touch the Home Icon.

## 5.2 Control Panel (Cont'd)



**Figure 5 – Control Panel**

The following sequence of events will occur from the current settings in Figure 5

1. Precool - the coil is inactive while the vial cools in liquid nitrogen for 1 minute. Samples must be precooled before grinding (10 minutes is recommended).
2. Cycle 1 - the coil is activated and the impactor grinds for 2 minutes at a rate of 10 cycles per second (20 impacts per second).
3. Cool Time - the coil is inactive for 1 minute between grinding cycles allowing coil and sample vial to cool down.
4. Cycle 2 - the coil is activated and the sample is ground for another 2 minutes.
5. Cool Time - the coil is inactive for 1 minute, as in step 3.
6. Cycle 3 - the coil is activated and the sample is ground for another 2 minutes.
7. Cool Time - the coil is inactive for 1 minute, as in step 5.
8. Cycle 4 – the coil is activated for the final 2 minute cycle. At the end of the final cycle the program is complete and the sample has been grounded for a total of 8 minutes.

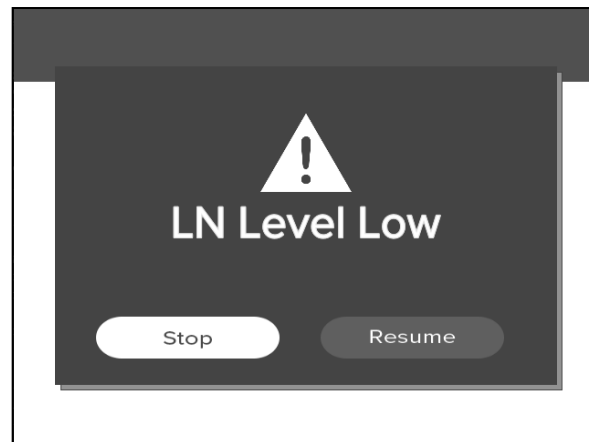
To change the settings of a selected field, press the minus (-) or plus (+) buttons on the **CONTROL PANEL** screen. The minus (-) button decreases the number displayed and the plus (+) button increases the number displayed.

### 5.2.1 Starting a Programmed Run

To run the program displayed on the **CONTROL PANEL** screen, touch run button. The Freezer/Mill can be started, stopped, or paused in the middle of a grinding program from the button selections to the right of the Run Screen (Figure 8).

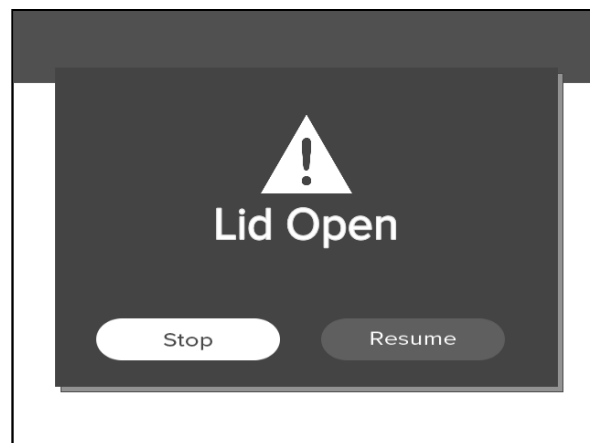
## 5.2 Control Panel (Cont'd)

Open the lid and place the loaded sample vial in Coil Vial Opening, then **SLOWLY** close the lid to keep the liquid nitrogen from splashing or spilling out the tub. Two additional loaded sample vials can be placed in the precooling basket while running the sample. By precooling the additional loaded sample vials, the PRECOOL time for these samples can be decreased or eliminated during the next run. After all vials have been loaded, lower the lid and secure the latch. If the liquid nitrogen in the tub is not at the correct operating level, then the screen will display LN LEVEL LOW (Figure 6). Add more LN and touch RESUME to begin operation.



**Figure 6** – LN Level Low Screen

If the lid is not completely closed and latched, the screen will display LID OPEN, as shown in Figure 7. To abort the program touch the stop button. To continue with the program touch the RESUME button after the lid has been completely closed. When the tub is filled with liquid nitrogen to the correct level, and the lid is securely latched, the STATUS BAR will turn **green** and begin counting down. The PRECOOL sequence will start indicating the beginning of the grinding program.

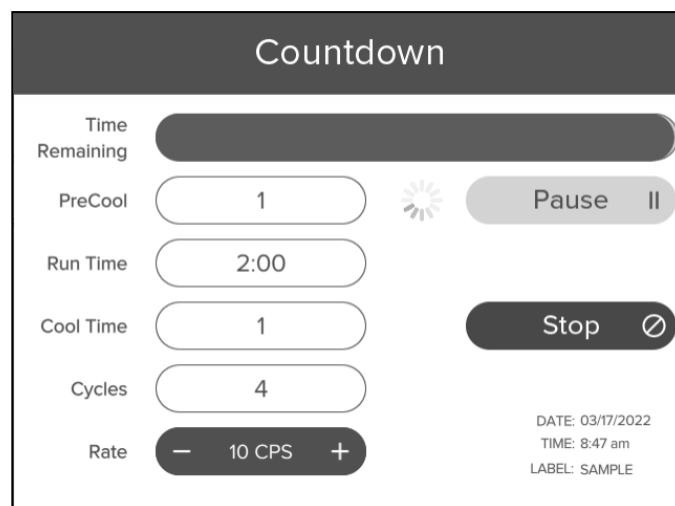


**Figure 7** – Lid Open Screen

## 5.2 Control Panel (Cont'd)

During the PRECOOL stage the TIME REMAINING line counts down the time for that stage in 1-second increments. To pause the program, touch the pause button. To continue the program touch the RESUME button. To abort the program, touch the stop button. To start a new program, touch the run button. When the programmed stage (e.g. PRECOOL) is complete a **green check mark** will appear next to that field. (Samples must be precooled)

Following the PRECOOL stage is the first GRINDING cycle stage. The RUNTIME line will display the time remaining in that cycle of the grinding stage. At the end of each grinding period a **green check mark** is placed next to that field. If there are 4 total grinding periods, the CYCLES line will countdown to "0".

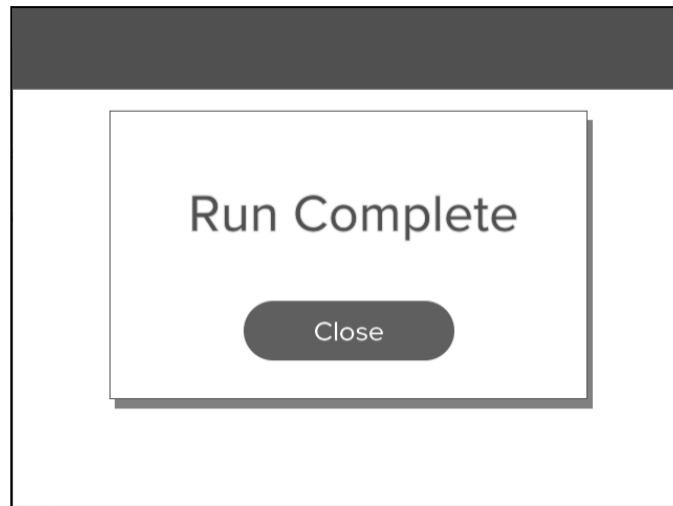


**Figure 8 – Run Screen during grinding**

As shown in Figure 8, the RUNTIME line indicates the 2 minute grinding period will begin after the Pre-Cool (1-min) is complete. Between any two grinding periods is the COOL DOWN stage. The COOL TIME line display, the time remaining in the COOL DOWN stage. The Cool Time for 1 minute is set to start as indicated by the yellow symbol. At the end of each Cool Time period a **green check mark** is placed next to that field. The Rate is 10 cps (cycles per second).

The STATUS BAR (top of screen) counts down the Time Remaining for the programmed run. The bottom of the screen displays the Protocol Name for the run. If no Protocol Name is assigned to the run as a Saved Protocol (Section 5.4) then the default name will be displayed as COUNTDOWN. When the grinding program has ended, the screen will display RUN COMPLETE (Figure 9). Touch the screen to return to the Control Panel.

## 5.2 Control Panel (Cont'd)



**Figure 9 – Run Complete Screen**

### 5.2.2 Adjusting the Grinding Rate

While in the **CONTROL PANEL** screen, the only parameter that can be modified during grinding is the RATE. It is adjusted by touching the minus (-) and plus (+) buttons next to the Rate field. Touching the minus (-) button decreases the rate by 1 cps at a time, while touching the plus (+) button increases the rate by 1 cps. Tap or hold the minus (-) or plus (+) buttons until the desired rate is reached.

Rate is the number of back-and-forth cycles per second (cps) completed by the impactor. During one cycle the impactor strikes both end plugs of the vial, therefore a rate of 10 cps is equivalent to 20 impacts per second. The factory default setting for the rate is 10 cps. The maximum grinding rate is 15 cps and the minimum is 5 cps.

### 5.2.3 Stopping or Pausing a Run

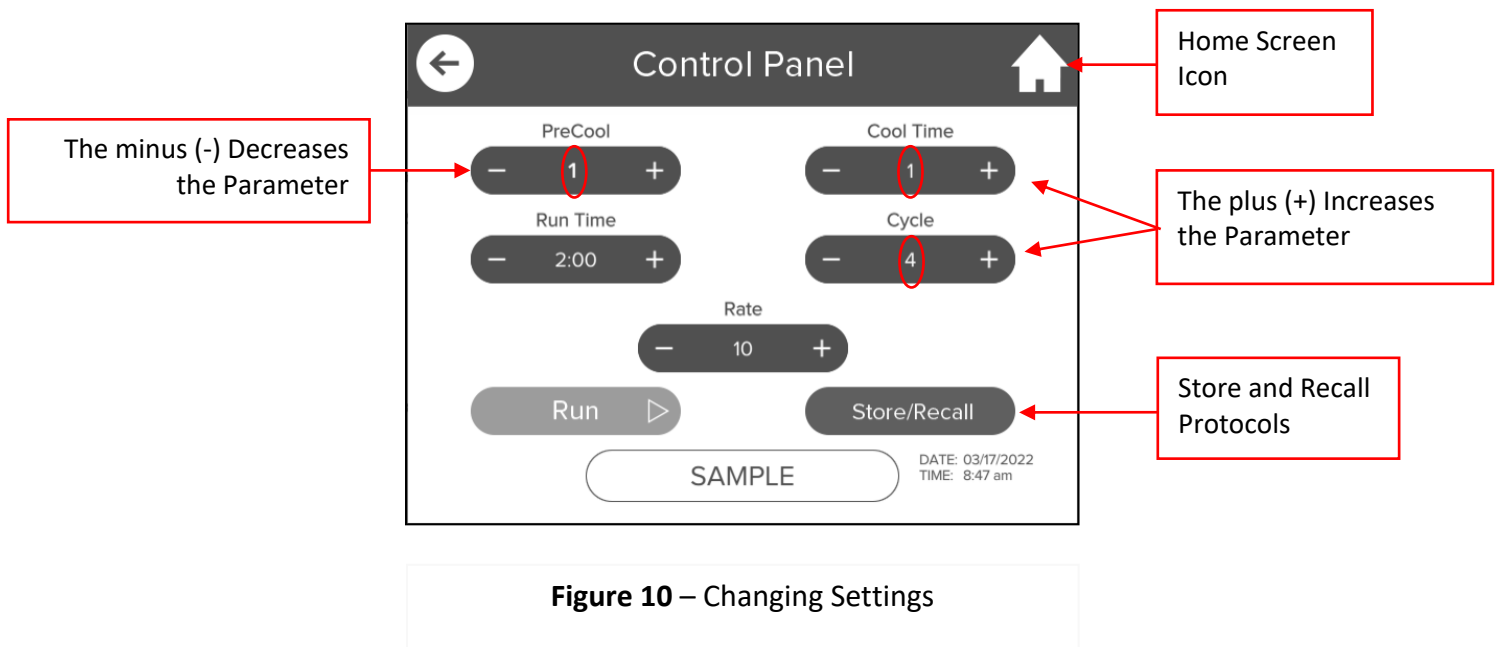
To stop a program, touch the stop button on the **CONTROL PANEL** screen and the program will end. If the run button is touched after the program has stopped then the entire program will start over.

To pause during a grinding program, touch the pause button. The **RUN** will hold the settings at that moment (elapsed time, cycle, etc.). To resume the program, touch the Resume button. This will restart the program at the point at which it was paused. Alternatively, pressing STOP will end the program.

### 5.3 Changing Settings

To change parameters (precool time, grinding time, cool down time, cycles, and rate), touch the plus (+) and minus (-) buttons on the **CONTROL PANEL** screen. The changes will appear in the gray area between the (-) and (+), as shown in Figure 10.

The CONTROL PANEL allows the user to change the parameters by touching the minus (-) and plus (+) buttons next to the field. Touching the minus (-) button decreases the parameter, while touching the plus (+) button increases the parameter. Tap or hold the (-) or (+) buttons until the desired number is reached. To run a program with the new settings, touch the run button.



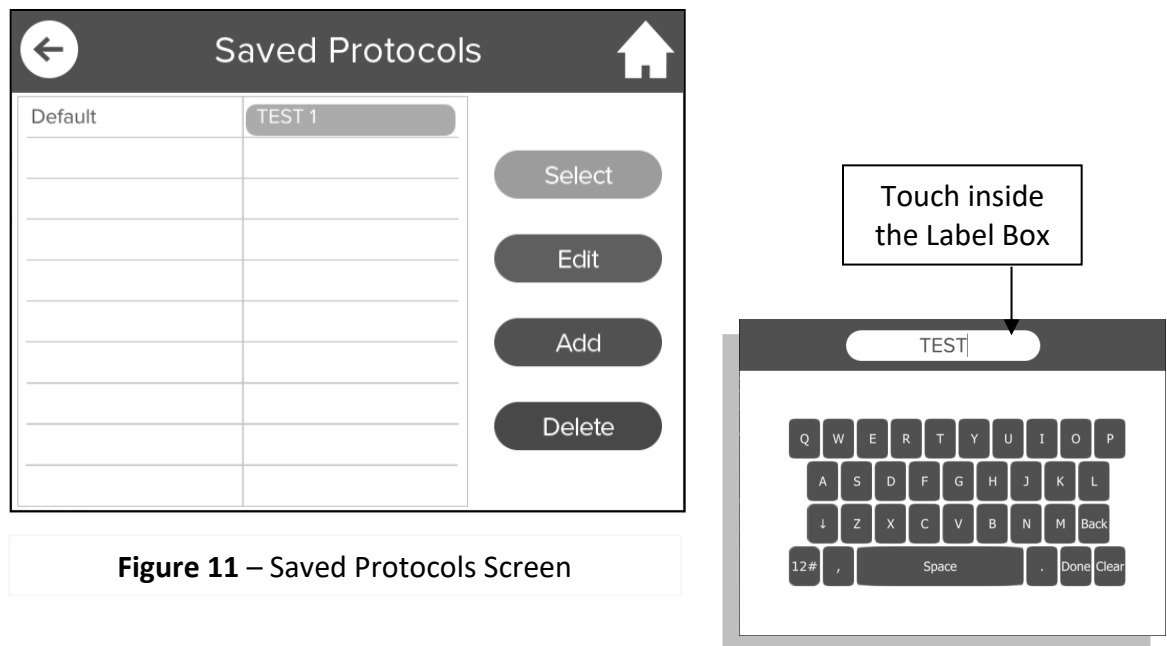
### 5.4 Saved Protocols

The **SAVED PROTOCOLS** screen is shown in Figure 11. In this example, three saved protocols have been stored and named (e.g. Hair, Bone, Soft Tissue). Up to 20 Protocols can be stored. To access or make visible additional protocols, or vacant protocol spaces (e.g. User Protocol) swipe up or down the touchscreen. This brings up vacant protocol slots on the **SAVED PROTOCOLS** screen.

A saved program retains the settings for number of cycles, precool time, run time, and cool time between cycles. Be sure to adjust the rate to the desired setting after recalling a stored program.

The Default protocol recalls the last programmed run setting that was not saved. The Default protocol can be changed without saving the new settings, as described in section 5.4.

## 5.4 Saved Protocols (Cont'd)



**Figure 11 – Saved Protocols Screen**

### 5.4.1 Storing a new program

To store the new program or to recall a stored program, touch the store/recall button on the Control Panel screen (Figure 5). In the **SAVED PROTOCOLS** screen touch inside the Label Box, as shown in Figure 12.

This will bring up the **KEYBOARD** screen, a simplified version of the standard keyboard for a computer. In addition to number and letter keys, this keyboard has standard symbol keys (#, %) and four function keys (SPACE, BACK, DONE, and CLEAR).

As the letters/numbers are touched, they appear above the keyboard in the Label Box with a centered cursor. To access the number and symbol keys touch the 12# button located bottom left on the **KEYBOARD** screen. To switch back to letter keys touch the ABC button (same button) bottom left on the **KEYBOARD** screen.

The Up/Down yellow arrow key on **KEYBOARD** allows the user to shift back and forth from upper case to lower case (the default is upper case). Touch Down arrow to switch to lower case. Or touch Up arrow to revert to upper case.

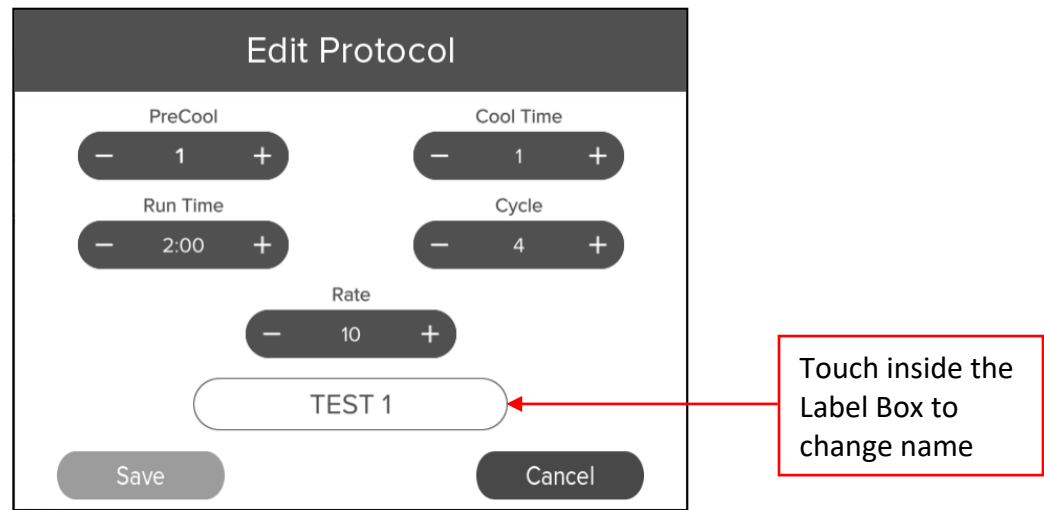
Touching the SPACE key advances the cursor one space. Touching the BACK key deletes one space. The CLEAR key deletes whatever has been entered in the Label Box.

Touch the DONE button to save the name shown in the Label Box. The label will appear as the name of the program in the **SAVED PROTOCOLS** screen (Figure 11).

## 5.4 Saved Protocols (Cont'd)

The CANCEL key does not change anything on the screen, but returns the display to the **SAVED PROTOCOLS** screen.

To run the newly saved protocol, touch the protocol to highlight the name. Then touch the LOAD button to send protocol to the **CONTROL PANEL** screen. Review the parameters and touch the run button to initiate the protocol.



**Figure 12** – Edit Protocol Screen

### 5.4.2 Delete Protocol

To delete a saved protocol, touch the protocol to highlight the name. Then touch the Delete button. A pop-up window opens confirming to delete this protocol. Touch YES to clear or touch NO to keep the saved protocol.



**Figure 13** – Delete Protocol

### **5.4.3 Edit Saved Protocols**

To edit a saved protocol, touch the protocol to highlight the name. Then touch the EDIT button. From the Edit Protocol screen change parameters and/or name, then touch the DONE button.

### **5.4.4 Recalling a program**

In the **SAVED PROTOCOLS** screen, touch the protocol to highlight the name. Then touch the LOAD button to send protocol to the **CONTROL PANEL** screen. Review the parameters. To RUN the protocol touch the run button to initiate the protocol.

## **6.0 OPERATION**

### **6.1 CG-400: Manually Adding Liquid Nitrogen Coolant**

Liquid nitrogen is manually added to the CG-400 Freezer/Mill using a portable Cryogenic Dewar. Fill a 10L or 15L Dewar with LN, open the lid and pour LN in the tub. For a larger capacity Dewar (20L). For a selection of Dewars visit [coleparmer.com/c/cryogenic-storage-dewars](http://coleparmer.com/c/cryogenic-storage-dewars). The CG-400 should be filled until the LN level reaches approximately 2 inches (5.1 cm) below the lip of the tub. If the liquid nitrogen is above this level when the lid is closed, LN can splash outside the tub. ***Always close the lid slowly to avoid splashing.***

Liquid nitrogen will most likely be available either in a large tank with a flexible steel hose, or in a smaller Dewar. The CG-400 Freezer/Mill typically consumes 10 to 15 liters of LN during initial cool-down, and another 4 to 6 liters per hour, depending on use.

Unlatch the lid and the gas cylinders will push it open. To unlock the latch, press down on the lid and pull the top of the latch forward and down. Disengage the bottom of the latch and the lid can be opened. The coil assembly is attached to the lid and will rise with it. The chamber gate is on the right side of the coil housing. To lower the gate to insert a loaded vial for grinding, lift the coil gate handle to the horizontal position and twist to align with the slot in the coil gate. Then pull the coil gate to the right to open.

Note that there are three different sizes of vial that can be run in the CG-400/CG-450 Freezer/Mill: Large, Mid-Size, and Small. Mid-Size vials must be run with a 6886 Adapter in the coil, and Small vials must be run with a 6807 Adapter in the coil. Large vials do not need an Adapter. See Section 4.4 for details.

With the vial or vials (and adapter) in place, loosen the coil gate handle a turn or two counterclockwise, align the handle with the slot, close the gate, and turn the handle clockwise until the gate is in snug contact with the vial or vials. To lock the gate, fold down the gate handle against the gate. ***Do not overtighten the gate, as this can crack the vial's center section.***

## **6.1 Adding Liquid Nitrogen Coolant (Cont'd)**

If the Freezer/Mill tub is empty, pour liquid nitrogen (LN) into the tub until it is approximately one-third full. Initially LN will boil off vigorously, but as the tub cools the boiling will subside. Add more LN gradually, to avoid splashing and boiling over, until the LN level is approximately 2 inches below the rim of the tub. When boiling is subdued, push the lid down gently until the bottom of the latch can be engaged. Pause if splashing becomes excessive. When the vapor stream has subsided, open the lid again and top off the liquid nitrogen in the tub, filling it to the mark on the inside wall of the tub. Insert a loaded vial or vials into the coil (if this has not already been done), and tighten and lock the coil gate.

Press the Freezer/Mill lid closed and latch it. **Caution: When the lid is closed and latched, nitrogen vapor which vents from the Freezer/Mill can displace the oxygen in a closed room and cause asphyxiation.** A grinding program can now be run, as outlined in Section 5.2.

## **6.2 Loading Samples into Freezer/Mill Vials**

### **6.2.1 Introduction and General Recommendations**

Start by choosing a sample that is typical of those to be ground on a regular basis. As a rule of thumb, the sample pieces should be small enough to circulate inside the vial as the impactor moves back and forth, and should take up no more than  $\frac{1}{3}$  to  $\frac{1}{2}$  the volume of the vial, with the impactor in the vial. Typical sample sizes for the different sizes of Freezer/Mill vials are shown below in Table 1.

In practice, the optimum parameters (sample weight and size, precooling time, grinding times and number of cycles, impactor rate, etc.) are customized to match particular samples.

Your own procedure for specific materials should be determined by trials; in practice almost no two materials grind alike.

In practice, the optimum parameters (sample weight and size, precooling time, grinding times and number of cycles, impactor rate, etc.) are customized to match particular samples. Your own procedure for your own materials should be determined by trials; in practice almost no two materials grind alike. Avoid overfilling vials as this greatly reduces grinding efficiency.

If at first the sample doesn't grind as intended, try these strategies:

- Reduce the overall weight of the sample.
- Reduce the size of the individual pieces to  $\frac{1}{4}$  or  $\frac{1}{8}$  inch (6 to 3 mm).
- Increase the precooling time. (15 minutes of precooling is about the maximum necessary for most samples to reach LN temperatures.)
- Grind longer. (20 minutes of actual grinding time is the maximum recommended for one run, due to the LN capacity of the tub, but you can top off the LN and repeat the run if necessary.)

**Table 1.** Typical Sample Sizes

Sample	Large Vial 6801, 6803, 6871	Mid-Size Vial 6881, 6883, 6885	Small Vial 6751, 6761, 6771, 6781S	Microvial 6757
Plant tissue, bone, muscle, etc.	20-50 g	5-20 g	2-5 g	100-300 mg
Polymer pellet	10-20 g	2-10 g	1-2 g	NA

### 6.2.2 Size of Sample Pieces

The size of sample pieces is important. For uniform results, most samples should be cut into pieces smaller than ¼ inch (6 mm). Very tough samples such as fresh bone should be cut smaller, down to ⅛ inch (3 mm). There are several reasons for this:

- The more irregular the size of the sample pieces, the more difficult it is to predict the results. Uniform procedures work better with uniform samples.
- Thin fibers and films can be very difficult to cryogrind unless they are cut into small pieces first. Wads of long fibers and crumpled balls of film take up extra room and can be elastic, even at LN temperatures.
- Tough samples in large pieces can be very difficult to grind, and in rare cases may damage the vial. A large, wedge-shaped piece of bone or a tough polymer can act as a wedge to push a moving impactor through the side of the polycarbonate tube.

### 6.2.3 Loading a Sample into a Vial at Room Temperature

Most Freezer/Mill samples do not have to be kept cold all the time, and can be loaded at room temperature. To load a vial, first assemble it halfway by pushing an end-plug into a center cylinder. Add the sample and impactor to the vial, and close it with the other end-plug. Shake the vial to make sure the impactor has room to move back and forth.

### 6.2.4 Loading a Cold Sample into a Vial and Keeping it Cold

Some samples must be kept cold, such as plant or animal tissue being prepared for RNA extraction. When a sample is cold, and must be kept cold throughout the grinding process, the loading procedure is more elaborate.

Assemble the center cylinder and blunt end plug, as in 6.2.3, then chill the end plug and about an inch of the cylinder in a shallow liquid nitrogen bath. The vial rack that comes with the CG-400/CG-450 Freezer/Mill can be placed in an insulated container and used to hold the vials upright during chilling. At the same time, chill the impactor separately. As soon as the submerged end of the vial is chilled, drop in the cold sample and then the chilled impactor. ***(Always wear insulated gloves when handling chilled vial components directly!)*** Now carefully insert the other end plug, still warm, into the warm end of the cylinder. The vial can now be carefully placed in the mill, or submerged in a liquid nitrogen bath outside the mill, as long as care is taken not to let the sample touch the warm end plug before it has cooled down.

## 6.2 Loading Samples into Freezer/Mill Vials (Cont'd)

***CAUTION:*** *Never force an end plug (warm or cold) into a polycarbonate cylinder (warm or cold). Polycarbonate shrinks when chilled, and can crack if stressed. If the cylinder has cooled to the point that the end plug fit is too tight, allow the cylinder to warm up before inserting the end plug.*

### 6.2.5 Loading a Very Small Sample into a Vial and Keeping it Cold

A variant of the loading technique in Section 6.2.4 can be used to grind very small samples with the open pre-chilled vial upright. Place the sample on the chilled end plug and add a small amount of fluid such as an extraction medium or water. The frozen fluid will bulk up the sample enough so it will circulate in the vial during grinding. To speed up this process, some users first prepare small “ice cubes” of the sample frozen together with 0.5 ml to 1 ml of fluid.

## 6.3 Loading a Vial into the Freezer/Mill

Before loading a vial or vials in the CG-400/CG-450 Freezer/Mill, put on cryogenic gloves. Unlatch and lift the lid of the mill, and open the coil gate. If Small or Mid-Size vials are being used, make sure the proper Adapter is in the coil. Pick up the vial(s) with a gloved hand or an Extractor/Vial Opener and insert the vial(s) into the coil. Close the coil gate and tighten it against the vials(s) by turning the gate handle clockwise, but do not overtighten the gate. Lock the gate by folding down the handle.

Additional vials can be placed in the precooling basket and carefully immersed in the LN tub on the right hand side (end which faces the coil gate) with the basket handle against the tub wall.

This allows additional vials to precool during the grinding process. Thus, when the next vial is to be run, the precool step can be skipped. ***Remove the basket from the LN with caution. Do not immerse a hand into the LN, even when wearing a protective glove. Use a pair of tongs to grasp the basket handle if it is below or near the LN level.***

Check the liquid nitrogen (LN) level and top it off if necessary. Gently close the Freezer/Mill lid, and latch it shut. At this point a grinding program can be run using the controls described in Section 5.

## 6.4 Removing a Vial from the Freezer/Mill

At the conclusion of the grinding cycle, unlatch the lid and let the gas cylinders lift it. Do not force the lid, but allow it to rise at its own speed. If the gas cylinders are too worn or chilled to lift the lid, lift the lid gently.

Remove the vial or vials from the coil, using gloves or an Extractor/Vial Opener. At this point either close the lid to conserve LN or insert another vial and begin another grinding cycle.

## 6.5 Opening, Emptying, and Cleaning Freezer/Mill Vials

### 6.5.1 Opening a Vial

To open the large vial, slip the open end of the 6804 Extractor/Vial Opener over the flanged end-plug, align the pegs in the end plug with the slots in the Extractor, and turn the knob clockwise until the end plug is drawn out. If the end plug is drawn out within a millimeter or two of the end of the center cylinder and stops, rock the 6804 Extractor gently to dislodge the end plug. Always be careful that the vial does not fall and spill its contents.

If the Extractor/Vial Opener jams with the end plug part way out, let the vial warm up before removing the end plug. Forcing out the end plug can damage the Extractor or break the polycarbonate center section. The best way to prevent the Extractor from jamming is to make sure both the Extractor and the threaded end plugs are dry and clean before use.

If the Extractor/Vial Opener jams when being threaded into a cold end plug, it is probably due to water condensing and freezing on the extractor screw. The water freezes when it contacts a cold end plug. If this happens, make a habit of wiping off the screw with a paper towel before each use. Note that the end of the screw is tooled with an "X" cut to help remove any ice that forms on the end plug, but this is not always effective. Chilled vials can also be opened easily and quickly with the optional accessory 6808 Extractor for Large Freezer/Mill Vials.

On rare occasions a flanged end plug will stick in the vial when cold, even if the Extractor screw and end plug threads are dry and clean. If this happens do not force the end plug out, as the vial and/or the Extractor may become damaged. Allow the vial to warm up. The vial will warm up gradually, wrapped in several layers of cloth or paper towels. Once the vial is warm, the stuck end plug can be drawn out by hand or with a pair of pliers. When using pliers to extract an end plug, rotate the end plug and pull gently, as if unscrewing a bottle-cap. Forcibly rocking the end plug from side to side can stress the polycarbonate tube and crack it.

***CAUTION: Pressure can develop inside a Freezer/Mill vial as it is warming. As pressure builds, an end plug can pop out with force and the sample can be lost. For this reason handle vials with care, and wrap them in paper towel. It is best to open chilled vials immediately after removing them from the Freezer/Mill.***

### 6.5.2 Emptying a Vial

As soon as the flanged end plug is removed from a cold vial, empty the contents of the vial into a suitable container, the quicker the better as condensation on a cold sample occurs rapidly. Often it is helpful to tap the closed end of the vial to release the ground sample. Remember that the impactor may slide out ahead of the contents or with them, and that the longer the sample is exposed to air, the more water it will acquire from condensation. Samples which were tacky, spongy, etc., at room temperature will also return to that state as they warm up, and may agglomerate.

## 6.5 Opening, Emptying, and Cleaning Freezer/Mill Vials (Cont'd)

The impactor will have to be separated from the sample and can be handled with gloved fingers, a strong magnet, tongs, or pliers. Tools without a good gripping surface are likely to slip off the impactor. 6870L Accessory Pack included a Magnetic Extractor (6791). This is a rod with a magnetic tip that can be used to remove the impactor from a vial before emptying the contents.

If some condensation on a cold sample is unacceptable, either wait for the sample vial to warm up before opening and emptying it, or open and empty a chilled vial in a glove box filled with dry nitrogen gas.

Small amounts of sample usually adhere to the vial's impactor, end plugs, and plastic cylinder. Brushing or scraping these surfaces of the vial can often recover this fraction of the sample. Again, speed is important to minimize condensation and other effects of warming.

If it is necessary to recover 100% of the sample, let the vial warm up and add some water or other liquid that will not affect the polycarbonate (see Section 6.5.3). Shake the vial, empty it onto some filter paper, and rinse again if necessary to recover the entire sample.

### 6.5.3 Cleaning the Vials

The Freezer/Mill vials may be superficially cleaned quickly and easily by placing them under running warm water. If the vial is cold a coating of ice will form on the steel parts, but will melt quickly as the water runs.

The plastic center cylinders for all vials, and the Poly-Vial end plugs and impactor capsules, are made of polycarbonate. ***While this polymer is very tough at low temperatures, it is sensitive to alcohol, acetone, and other organic solvents, and should be cleaned only with soap and water.*** A mild bleach solution will control organic contamination. Polycarbonate can be autoclaved, but this will weaken it.

Before re-using polycarbonate cylinders, ***always*** inspect them for cracks or other damage. They may last for dozens or hundreds of samples, but as soon as they begin to crack they should be discarded. Liquid nitrogen can enter through cracks and vaporize when vial is removed from Freezer/Mill. As the vial warms vaporized LN will build pressure and an end plug can pop out with force so the sample may be lost.

If the sample adheres to the steel end plugs and impactor, they can be cleaned with water and soap or detergent, or with organic solvents. If they must be disinfected or cleaned of any organic residue, they can be washed with bleach or chemical cleaners or autoclaved, but should always be dried immediately after use. The steel parts in the 6751, 6801, and 6881 vials are made from 440C Stainless Steel, a magnetic stainless steel, which is corrosion-resistant but may rust to some extent if left in contact with water for too long. All stainless steel parts should be dried after washing.

### **6.5 Opening, Emptying, and Cleaning Freezer/Mill Vials (Cont'd)**

The steel parts of the chromium-free 6771, 6871, and 6883 vials can also be washed, disinfected, or autoclaved. **Do not wash with bleach or chemical cleaners.** Chromium-free steel is not rust-resistant and must always be dried immediately after washing. Store Cr-Free Vial parts in a sealed bag with a desiccant.

Rust on steel Freezer/Mill parts can be removed by scrubbing them with steel wool or an abrasive cleanser. If rusting persists, store the parts in a sealed bag with a desiccant.

### **6.6 Checking the Liquid Nitrogen Level in the CG-400**

The LN should be topped off when needed as a matter of routine. If the programmed grinding cycle includes more than twenty or thirty minutes of actual grinding time, the nitrogen level should be visually checked at about that point and more added if needed. A cumulative grinding time of more than thirty minutes per run is not recommended, as after that much grinding the LN level is close to the point where the LN sensor will shut down the mill. However, if the mill must be run for an extended time, and the LN sensor stops the grinding program, the mill can be refilled with LN and the grinding program resumed where it stopped.

## **7.0 CG-450 AUTO FILL SYSTEM**

### **7.1 Description**

The CG-450 Auto Fill System automatically transfers liquid nitrogen (LN) to the CG-450 Freezer/Mill during operation, making hand-filling unnecessary. The Auto Fill mechanism includes a cryogenic valve linked to LN sensors in the tub. When the LN level is low, the valve opens during Precool or cooling stages to let LN into the tub. The Auto Fill System as supplied also includes a safety valve for the LN transfer hose.

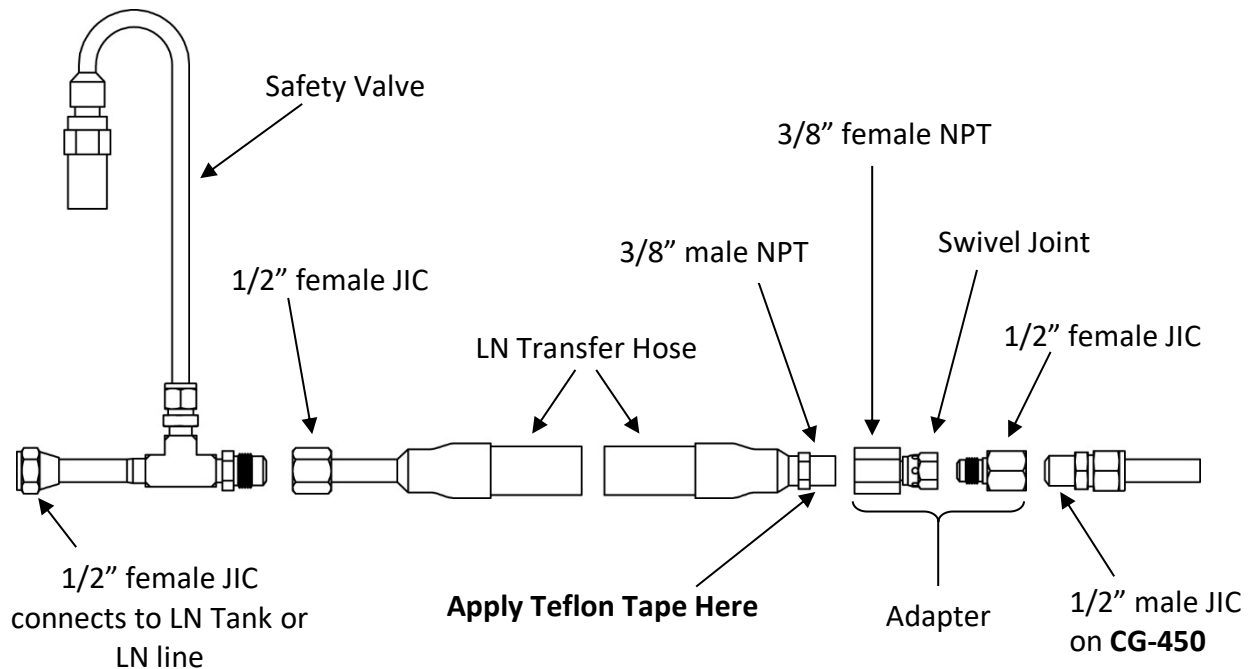
LN transfer hoses can vary with the LN supply tank or line and are therefore not included with the CG-450 Auto Fill System. Four-foot and six-foot transfer hoses of standard design (part numbers 6906 and 6907) can be purchased from Cole-Parmer, or custom transfer hoses can be designed and supplied by the user. The LN inlet is a male ½ inch JIC fitting placed low on the right side of the Freezer/Mill, so the outlet of the hose requires a female CGA295 fitting. The 160 liter and 240 liter LN tanks used by many of our customers generally have a male 3/8 inch (9.5 mm) NPT outlet, Cole-Parmer transfer hoses come with a matching female 3/8 inch NPT fitting. Excessively long transfer hoses are not recommended.

**Note:** LN tanks come in two types, high-pressure and low-pressure. Some LN tanks have valve systems to switch from low pressure to high pressure. **Always use a low-pressure LN supply, with a delivery pressure of 20-22 psi.**

## 7.2 CG-450 Liquid Nitrogen Connection

Install the safety valve on the outlet of the tank or LN line, with the gooseneck tilted up and the valve outlet down (see Figure 14). Then attach the LN transfer hose to the safety valve, and the other end to the inlet on the Freezer/Mill. The safety valve should be higher than the LN inlet on the Freezer/Mill. The purpose of the safety valve is to have an outlet for nitrogen vapor in case the mill and supply tank valves are both shut, and pressure builds up inside the hose.

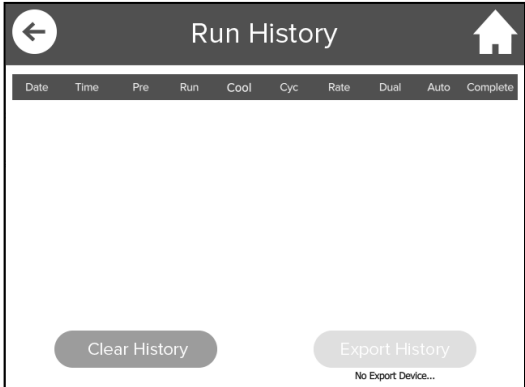
When installing the safety valve and transfer hose, use Teflon plumber's tape on all joints, and tighten the nuts with a wrench. If the joints leak, tighten them further and/or use more tape.



**Figure 14** – Liquid Nitrogen Connection (LN Transfer Hose Sold Separately)

8.0 Run History

To recall Run History touch the Run History button. The Date, Time, and Run Protocol data are stored on this screen, as shown in Figure 15. The run information can be exported to other computer devices via the USB Port located on the back of the Controller. To export run data touch the Export History button at the bottom right of the screen. To clear Run History or delete Run History permanently from data storage touch the Clear History button at the bottom left of the screen. To return to the previous screen touch the back arrow button at the top left corner of the screen.

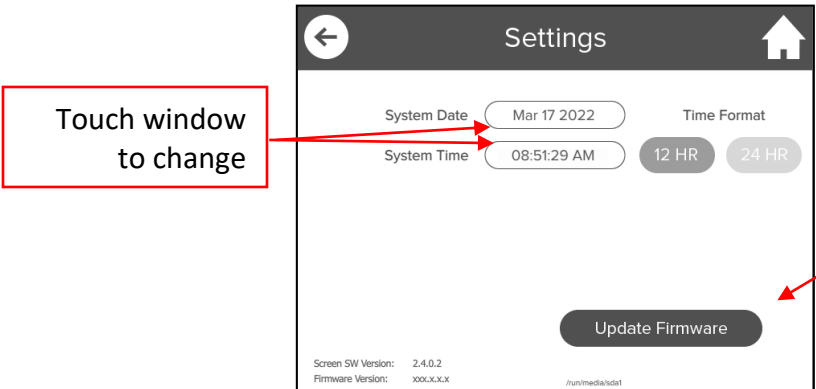


Note, the Export History button will have a dimmed or muted green appearance which indicates it is inactive. After a flash drive or USB cable (connected to computer device) is inserted into the port the button will turn a brighter green indicating it is active.

Figure 15 – Run History Screen

9.0 System Settings

To access the Settings Screen touch the Settings Icon on the Home Screen (Figure 4). The System can be upgraded, Diagnostics view/exported, and files exported (Run History, Saved Protocols) and saved protocols imported, as well as the time display changed from 12-hour to 24-hour, and Time and Date can be entered or changed, as shown in Figure 16. Touch inside window to change the Date or Time. A pop-up window opens to enter Time or Date. Touch the SET button to confirm the change. Select the 12 or 24 hr. time, which will be displayed on the Run screen. Touching the back arrow button returns the user to the previous screen.



**Note:** System Update button appears only when an external flash drive is connected to the USB Port (back of controller).  
**[System updates furnished only by COLE-PARMER, if necessary]**  
To update system, connect COLE-PARMER flash drive. After unit detects device, touch button to upgrade system.

Figure 16 – Settings Screen

## **10.0 MAINTENANCE**

The CG-400/CG-450 Freezer/Mill has been designed to provide trouble-free operation over a long period of time. To ensure proper performance it is very important to keep the unit clean. When the Freezer/Mill is at room temperature, any spilled powders or liquids should be wiped up immediately. The internal tub should be wiped clean with a damp cotton cloth after every use.

This should prevent the buildup of any powders, mold/mildew, or other residue over the life of the unit. If any samples, powder materials, or liquids are spilled inside the unit during a sample run, wait until Freezer/Mill is finished for the day, the liquid nitrogen has evaporated, and the mill has warmed up to room temperature before attempting to clean it. Once the unit has attained room temperature, wipe down the unit as indicated previously. After every period of use, the mill will become wet with condensation due to the Freezer/Mill picking up moisture from air when it is cold.

To maintain the exterior of the unit, first disconnect the Freezer/Mill from its electrical source. Then lightly spray with a mild window cleaner or similar product and wipe the unit down. If the CG-400/CG-450 Freezer/Mill requires service, please call Cole-Parmer.

## 11.0 TROUBLE-SHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	POSSIBLE SOLUTION
<b>The unit does not turn on.</b>	No current.	Make sure power cord is plugged into outlet and inlet.
	No current.	Check outlet for power cord and correct as required.
	Power switch not turned on.	Press power switch to ON position.
	Circuit Breaker tripped.	Turn unit OFF then ON Call Cole-Parmer for service or repair.
<b>Screen displays “LID UP”.</b>	Safety interlock switch not engaged.	Close the lid and latch.
	Lid is not closed completely.	Push the lid closed and latch into place.
<b>Screen displays “LN LEVEL LOW”.</b>	Liquid nitrogen sensor detects inadequate liquid nitrogen level.	(CG-400) Pour more liquid nitrogen into the tub. (CG-450) Check the LN Tank has LN.
<b>Coil gets very warm.</b>	Low liquid nitrogen levels.	Pour more liquid nitrogen into the tub.
<b>Impactor doesn’t move back and forth.</b>	Water in vial has frozen impactor.	Remove vial and replace vial and contents with a dry unit or RAP ends of the vial on counter to free impactor.
	Impactor is magnetized.	Remove impactor, turn end-for-end, or demagnetize impactor and re-insert.
	Too much sample is in the vial or sample pieces are too large	Remove some sample from the vial and start again
<b>Flanged End Plug doesn’t come off cold vial.</b>	End plug/tube joint too tight when very cold.	Allow vial to warm up.
<b>Blunt end plug doesn’t come off room-temperature vial.</b>	Hard to grip	Warm vial under warm water. Use wide-jaw pliers if necessary.
<b>6804 Extractor jams.</b>	Water on extractor screws or bell.	Dry extractor before each use.
	Ice in threaded hole in end plug.	Dry end plug thoroughly before using.
<b>Plastic center cylinder cracks.</b>	Cylinder cleaned with alcohol or other organic solvent.	Use bleach, detergent, and water to clean cylinders.
	The vial is assembled when chilled.	Never force end plug into a cylinder.
	Sample pieces acts as wedge.	Reduce the size of sample pieces. Make sure there are no sample particles on cylinder ends.

**12.0 WARRANTY**

Cole-Parmer® guarantees its products against defects in materials or workmanship for one year from the date of original shipment. Repairs, replacements, or parts are guaranteed for 30 days or for the remaining original warranty period (whichever is greater) for the item that was repaired or replaced. Items not produced by Cole-Parmer® carry the manufacturer’s warranty only.

Items not produced by Cole-Parmer carry the manufacturer’s warranty only. CG-400/CG-450 Freezer/Mill wear parts include the coil.

<b>Wear Parts</b>	
Part No.	Description
40256	Solenoid Coil

The customer pays return freight for warranty claims. If the warranty claim is valid, Cole-Parmer® will pay return freight to the customer. However, Cole-Parmer® reserves the right to judge whether a malfunction during the warranty period is due to defects in materials or workmanship, or to wear, negligence, or misuse.

**12.1 Product Specifications**

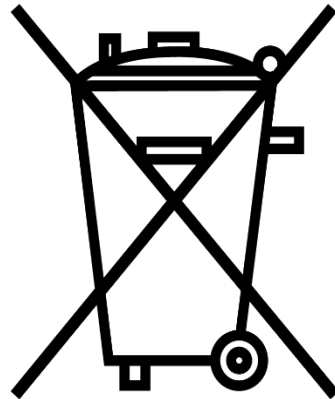
Every effort has been made to provide complete and accurate product operating information in this manual. However, since specifications are subject to change without notice, changes may be made from time to time to improve the performance, reliability, and function of the product. Therefore, slight changes that are not reflected in the current illustrations should be considered minor and inconsequential for the purposes of this operating manual.

**12.2 To Arrange a Return Shipment**

We want you to be satisfied with your purchase from Cole-Parmer®. Please bring any problem to our attention, but please DO NOT RETURN any item before contacting us for a Return Authorization Number and instructions. Unauthorized returns will be refused. The cost for all return transportation is the responsibility of the customer. Credit for returned merchandise will be issued only after goods have been received and inspected. Returned goods are subject to a 25% restocking charge.

**13.0 INSTRUMENT DISPOSAL**

In accordance to the EU Directive 2012/19/EU covering Waste Electrical and Electronic Equipment, all equipment with the disposal symbol must not be disposed of with general waste. (See Figure 17)



Disposal Label is located on the back of unit.



**Figure 17 – Disposal Symbol**

Throughout the European Community, guidelines regarding disposal regulations may vary from territory to territory. Please contact the national legislation or local authority for more information on proper disposal of all equipment with this symbol.

**14.0 CONTACT US**

**Repair Service**

Phone: 1.732.623.0465

Cole-Parmer SamplePrep  
65 Liberty St  
Metuchen, NJ 08840  
US

**Attn:** Service and Repair  
Please include RA Number on the shipping label.



65 Liberty Street  
Metuchen, NJ. 08840  
USA

Phone: +1.732.623.0465  
Email: [sampleprep@coleparmer.com](mailto:sampleprep@coleparmer.com)  
Web: [cpsampleprep.com](http://cpsampleprep.com)