



For research use only

# *cryoPREP System*

## **USER MANUAL**

A system designed and developed for tissue sample  
Collection, Storage, Pulverization,  
and Transfer in a cryo-environment

Product Name: cryoPREP System

Product Number: CP02



### UNIVERSAL PRECAUTIONS

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Blood borne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A3 Clinical Laboratory Safety; Approved Guideline - Third Edition, published 2012, ISBN 1-56238-797-9
- Clinical and Laboratory Standards Institute (CLSI), M29-A4 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Fourth Edition, published 2014, ISBN 1-56238-961-0
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Blood borne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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Covaris, Incorporated  
14 Gill St, Unit H  
Woburn, Massachusetts  
01801-1721 USA

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## **Warnings**

### **For safety of operating personnel:**

1. Make sure that the equipment is properly grounded. DO NOT operate if it is not properly grounded.
2. The unit is equipped with a power plug appropriate for the destination country. DO NOT, under any circumstances, remove the grounding prong from the power cord.
3. DO NOT attempt to operate the equipment without lid in the DOWN position. If there is any indication that the Safety System is not functioning properly, DO NOT operate the equipment and contact Covaris immediately.
4. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

### **To prevent damage to the equipment:**

1. The instruments are designed to operate in ambient laboratory conditions e.g., 19°C to 25°C (66°F to 77°F). DO NOT operate the instrument in a cold room environment. Establish a standard of operation and maintain the equipment as described in Maintenance (see section 6.0) of this manual.

## Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery. Any component which proves defective during the stated period will be repaired free of charge or replaced at the sole discretion of Covaris, F.O.B., Woburn, Massachusetts, provided the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement with telephone support from Covaris personnel. If Covaris personnel are required to perform on-site repair, all travel costs are paid by the customer. A limited warranty as specified may apply to certain components of the equipment.

### Warranty Exceptions

This warranty is void if failure of the software or hardware has resulted from accidents, abuse, improper maintenance, or repair, or misapplication by the customer. It is also void if damage is caused by any unauthorized attachments or if modifications are made to the equipment. Removing or tampering with the Safety Enclosure will void the warranty, and the customer will assume all liabilities.

This warranty is limited to the original purchaser and is not transferable.

The software will perform according to the accompanying written materials and the medium on which the software is delivered is free of defects in materials under normal use and service. The warranty is void if damage has resulted from third party software not intended for use with the system.

The high power focused transducer is designed to give maximal mechanical energy output in water. Permanent damage to the transducer and electronic circuits could result if the transducer is operated without water. Operation of the system without water in the water bath voids the warranty.

CONTACT COVARIS, INC. SHOULD YOU HAVE ANY QUESTIONS CONCERNING EQUIPMENT.

### Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the service and support necessary so that the customer can operate the equipment successfully. Extended warranties are available at the end of the original 12 month warranty period.

Services included with the original purchase of the system are:

**Installation and Training** – Setup and installation of the equipment and operator training can be purchased at time of initial acquisition of the equipment. Both will be performed by a qualified Covaris service representative. One half day will be scheduled to perform the installation and on-site training.

- The operators' training will include valuable hands-on time with the equipment.
- Preventative maintenance and troubleshooting tips will also be covered.

**Technical Support** – On-going assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- **Telephone**
  - United States: Tel: +1 781 932 3959 during the hours of 9:00am to 5:00pm, Monday through Friday, Eastern Standard Time (EST), Greenwich Mean Time (GMT-05:00)
  - Europe: Tel: +44 (0) 845 872 0100, during the hours of 9:00am to 5:00pm, Monday through Friday, Greenwich Mean Time
- **E-mail** queries to [techsupport@covaris.com](mailto:techsupport@covaris.com) or [applicationsupport@covaris.com](mailto:applicationsupport@covaris.com)

**Parts Replacement** – Replacement of parts (excluding consumables) for normal operation of equipment are provided on a priority basis. Shipping charges are included. Failure due to accident, abuse, or improper operation is not covered.

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## 1.0 Introduction

### 1.1 Overview of the Manual

This manual contains operation and service instructions for the *Covaris cryoPREP™ Series Instrument CP02*. It contains background information essential to the proper use and care of this equipment.

Should any unforeseen problems occur with the normal operation of the equipment, contact Covaris Technical Support immediately.

The following definitions apply in this manual:

**NOTE:** Inconvenience if disregarded.

**CAUTION:** Equipment damage may occur.

**WARNING:** Personal injury may occur.

### 1.2 Purpose of Equipment

Covaris developed a product and associated processing equipment to reduce the time and operator involvement required to reproducibly pulverize a solid tissue sample and transfer the contents to a tube for downstream processing, while maintaining low temperatures (-70°C or colder).

The patent pending cryoPREP™ (CP02) system is based on the effective pulverization of biological samples in a cryo-environment (reference 4), enabled by the freeze-fracture properties of the material to be pulverized. There is a relationship between the temperature of the sample and both the brittleness and the efficiency of the pulverization -- when the sample is colder, the following benefits occur:

1. The pulverization efficiency is improved resulting in smaller particles or powders. This may result in higher recoveries of target molecules.
2. The sample hardness is increased, making the samples easier to fracture. For example, pulverization of liver samples may be adequate at -70°C, whereas, pulverization of mineralized bone samples may require -80°C or colder.

The disposable component, called the tissueTUBE™, is a single-use design that eliminates cross-contamination. The tissueTUBEs are constructed of engineered polymers. It is used with a hammer / anvil means; with the automated apparatus CP02 to generate an impact force. These components when used together comprise the cryoPREP system.

Covaris' single-use, closed-tube low temperature preparation system is designed to both reproducibly pulverize solid tissue samples and to transfer the pulverized material to a homogenization vessel while at cryogenic temperatures. The objective is to utilize the brittleness of biological samples frozen to subzero temperatures to facilitate the fracturing of the bulk samples into smaller particles. By increasing the tissue (or sample) surface area and breaking up the extra-cellular matrix, extraction efficiency of target biomolecules is improved. When utilized in conjunction with Covaris' acoustic homogenization products (S-Series and E-Series), the sample homogenization time is reduced and the acoustic energy can process larger tissue masses. This system of freeze-fracturing in a self-contained vessel may also improve other non-acoustic-based homogenization and/or extraction techniques.

### 1.3 Description of System Components

The cryoPREP system is composed of four components:

- **cryoPREP pulverizer (CP02)**– An automated apparatus to transfer reproducible impact forces to a chilled, flexible tissueTUBE.
- **tissueTUBE** (cryogenic pouch) - A single use, sample pulverizing and transfer tube designed with a threaded cap for rapid transfer of contents to screw-thread culture tubes. The tissueTUBE may be directly immersed in liquid nitrogen and may be stored at -80°C. This comes in three sizes (see Appendix B):
  - TT05 for samples of mass < 50 mg
  - TT1 for samples of mass < 1 gram
  - TT2 for samples of mass < 2 gram
- **tissueTUBE Plug** (screw-thread cap) - A single use, plug with o-ring designed for the tissueTUBE for storage of tissue samples. This also comes in three sizes:
  - TT05-P for use with TT05
  - TT1-P for use with TT1
  - TT2-P for use with TT2
- **tissueTUBE Holder** – A holder that fits into the chamber to hold either the TT1, TT2 or TT05 tissueTUBE. Each holder is clearly labeled to avoid confusion.

**NOTE:** A tissueTUBE holder must be inserted prior to operation. The TT05 insert fits into either a TT1 or TT2 holder.



Figure 1

### 1.4 Intended Use

The cryoPREP System is intended as a general-purpose device to be used in a laboratory environment. No claim or representation is made for its use to identify any specific organism or biomolecule.

The cryoPREP system, and specifically the tissueTUBE, provides a system for rapid pulverization and transfer of solid biological specimens for analytical testing.

The tissueTUBE is designed for biological samples with a three-dimensional volume.

For example, some standard mammalian tissues which are appropriate for use include:

- Liver, Kidney
- Skeletal Muscle, Heart
- Dermal, Lung
- Brain, Adipose

Samples such as cell culture pellets are not appropriate.

The TT1-XT and TT05-XT are special tissueTUBEs, designed to withstand greater stress at cryogenic temperatures for harder tissues. The TT1-XT, TT05-XT, and the TT2 are all constructed with thicker pouch material that may be used for:

- Bone (in TT1-XT mass may be limited to <500mg)
- Seed
- Plant stem material

XT tubes may also be used with softer tissues, although most tissues are appropriate for the standard tissueTUBE.

**NOTE:** Do not use any tubes in the cryoPREP other than the TT05, TT05-XT, TT1, TT1-XT, or TT2 tubes.

## 2.0 Unpacking and Installation

### 2.1 Check for damage

The cryoPREP system and its accessories are delivered in one carton. The shipping company is responsible for damage to the equipment during shipment. If damage has occurred, notify the carrier immediately to establish proper basis for a claim.

### 2.2 Installing the cryoPREP Instrument

Place the cryoPREP system on any surface acceptable for laboratory instrumentation. Position the instrument away from direct sunlight and sources of heat and cold. Ensure that the user can easily reach the power cord. The room temperature should be between 19°C and 25°C.

### 2.3 Installing the tissueTUBE Holders

Unplug the unit and raise the cover. Rotate the retaining lever 90°. (See red circle in Figure 2)

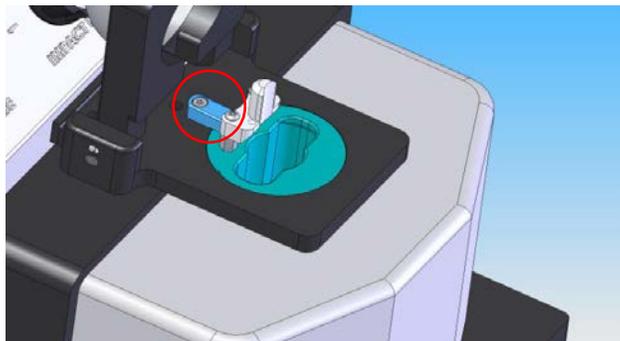


Figure 2

Insert a tissueTUBE sample holder carefully into the round opening. The holder will be labeled as TT1 or TT2. Rotate the lever 90° to keep the holder in place.

### Equipment Supplied by User

- Culture Tube. This is a biologically inert threaded, hard, borosilicate, round bottom glass tube with a writing patch and is suitable for efficient acoustic homogenization and extraction processes. The low cost tubes come with a liner-less polypropylene screw cap. This “transfer tube” may be used for further sample processing and storage. See Appendix B for appropriate sizes.

**NOTE:** Prior to sample processing, the culture tube serves as a cap for the tissueTUBE. Label the culture tube.

- ‘Cryogenic’ means one or more of the following:
  - Dry ice
  - Mechanical freezer (-80°C)
  - Dry ice alcohol bath
- Cryogenic gloves are recommended
- Forceps or tweezers

## 3.0 Safety Features and Precaution

### 3.1 Precautions

The cryoPREP system is designed to prepare tissue samples for bioanalytical testing. Accordingly, use of the cryoPREP system places a responsibility upon supervisory and safety personnel to ensure adequate training of operators as to the safe and effective use of the apparatus.

### 3.2 Hazards

The Covaris cryoPREP (CP02) automated system is not explosion-proof and should not be used in a potentially explosive atmosphere.

The CP02 is designed with a safety interrupt to prevent operation if either the sample cover lid is open, or if the clear front cover is removed.

### 3.3 Safety Information

The CP02 system is designed to fracture frozen biological samples. As such, the use of the system requires safety precautions appropriate for handling materials, samples, and components at subzero, cryogenic temperatures. For example, this may include the use of liquid nitrogen.

**CAUTION:** The system is not designed for use with infectious samples. For infectious samples, perform all processing in a biosafety containment hood.

Gloves are required, and cryogenic gloves are highly recommended for handling some of the system components and the tissueTUBE.

**CAUTION:** As the pulverization impact generates sound, safety earplugs, or ear protectors may be needed.

Because the tissueTUBE is subjected to cryogenic temperatures, when the relative humidity is high there will be condensation frost on the screw threads. The condensation will not adversely affect the pulverization performance as long as the “transfer tube” twists freely into the opening of the tissueTUBE. The TT2, TT1 and TT05 should be affixed to the “transfer tube”; and then opened a quarter turn to vent.

## 4.0 Controls

### 4.1 Front Panel

POWER BUTTON – Lit when power is on.

IMPACT LEVEL KNOB – Increasing force of impact from 1 to 6.

IMPACT ACTIVATION BUTTON – Lit when instrument is ready to impact.

## 5.0 Protocol

### 5.1 Instructions for use

As sample preparation protocols are diverse, individual protocols must be developed for specific laboratory applications, and as such, specific equipment may vary for different applications. It is recommended to practice a couple of times prior to processing samples for analysis.

Important areas to keep in mind prior to starting:

- **Cryogenic Source** - The following may be used to condition the samples prior to sample pulverization:
  - Dry ice (preferred)
  - Dry ice alcohol bath
  - Mechanical Freezer (-80°C)
  - Liquid nitrogen

**NOTE:** Dry ice is preferred over liquid nitrogen as it is less hazardous for the operator to handle.

For example, samples may be stored in the tissueTUBE in a freezer or the tissueTUBE may be transferred from the freezer on to dry ice (either packed in or laid horizontal on). In addition, liquid nitrogen may be used to increase sample brittleness immediately prior to impact pulverization.

- **Sample processing tube and cap** - The standard system requires the use of a culture tube affixed to the tissueTUBE. See Section 2.3. The TT05, TT1 and TT2 use different sized culture tubes. See Appendix B.
- **Labeling Strategy** – The glass culture tube has a white, writing patch. A barcode and/or other labels may be affixed to the culture tube to track the sample. The tissueTUBE pouch material is not designed for labeling as the immersion in liquid nitrogen results in an extreme thermal shock to the adhesives typically used for labels and the flexibility of the pouch at cryogenic temperatures allows inks to be dislodged from the surface. However, the white portion of the tissueTUBE may also be written on with a permanent marker pen.
- **Freezing Strategy** – The tissueTUBE can be directly laid horizontally on the dry ice. It may also be immersed directly into liquid nitrogen or a dry ice alcohol bath, therefore, a vessel to contain the fluid is required.
- **Sampling Strategy** – The number and type of samples will influence sample collection and thermal stabilization. For example, RNA extraction requires immediate thermal stabilization of the fresh sample and, as such, will require immediate immersion of the sample (i.e., in the tissueTUBE) into a cryogenic environment. This requires quick sample processing; liquid nitrogen is recommended. However, not all samples require rapid procurement, thermal stabilization, pulverization, and transfer. It is the responsibility of the user to determine whether or not liquid nitrogen is required. In some applications, using a mechanical freezer may be adequate.
- **Impact Force** – The force of the impactor may be varied. For example, small mass tissue samples (e.g., biopsy) that do not need the full force generated from the system may be

processed at impact level 1. Alternatively, with large samples it may be appropriate to use 3 or 4 impact level.

**NOTE:** The mass of the sample and the degree of connective tissue will determine the impact force. For example, 100mg of liver will require low impact while 500mg of muscle will require high impact.

As the actual impact pulverization process time is of a short duration (e.g., less than 1 second), some applications may be very quick. For example, an experienced operator may be capable of quickly loading and unloading the tissueTUBE and, therefore, frozen tissue samples may be easily pulverized in less than 10 seconds.

**NOTE:** After the sample has been pulverized, it is possible to affix the plug and use the tissueTUBE as a storage vessel. Aliquots of the pulverized sample may be removed for analysis.

In addition, some sample pulverization may not require the thermal stabilization of a freeze fracture protocol. For example, the disruption of dried seed material may not necessitate subzero conditions, however, the controlled impact force and the closed vessel, with no direct contact, process may be beneficial.

## 5.2 Procedure for preparing pulverized tissue

### ***Sample loading into the tissueTUBE***

tissueTUBEs are available in three sizes, TT05, TT1 and TT2. See Appendix B. There are two parts to the tissueTUBE; a threaded hub to affix the culture tube and a flexible pouch to hold the sample. The freshly procured samples are placed in the tissueTUBE as follows:

1. While holding the tissueTUBE, insert the sample specimen with forceps or tweezers through the opening and into the flexible pouch.
2. Ensure the sample is inserted in the center of the pouch; this is the impact zone for the pulverization.

**CAUTION:** The sample should not be touching the seams of the pouch as the impact process flattens the sample; the sample needs room to expand during flattening. The sample should be in the center of the pouch.

3. After the sample is loaded, affix the appropriate size Plug or culture tube to the tissueTUBE
4. While holding the plug or the culture tube, immerse the flexible pouch portion of the tissueTUBE into a cryo-environment (e.g., dry ice). The sample will be frozen in seconds.
5. After the sample is frozen, it may be stored at -80°C or may be used immediately by placing the tissueTUBE directly on dry ice (horizontal and covered recommended) prior to pulverization.

### ***Sample loading into the automated cryoPREP Pulverizer***

1. To pulverize the frozen sample, first remove the plug (if installed) from the tissueTUBE. Attach a glass tube to the tissueTUBE. It is preferred to have a chilled tube; although at this stage it is not necessary. For the TT2, TT1 and TT05, loosen the tube ¼ turn for venting

**CAUTION:** Before impact, loosen the “transfer tube” ¼ turn to prevent pressure buildup inside the TT05, TT1&TT2. If the tissueTUBE is processed with pressure buildup, it could rupture.

2. Orientate the tissueTUBE in a vertical position with the tissue pouch on the bottom and the glass tube on the top. This allows the pouch of the tissueTUBE to be inserted in a vertical position into the opening between the impacting hammer and the stationary anvil of the CP02 device.

**CAUTION:** Do not install with culture tube (glass) in the down position. The CP02 will shatter the glass.

3. Directly facing the front of the Impactor, raise the sample holder cover. With a gloved hand, insert the previously frozen tissueTUBE into the CP02 by orientating the wide base of the pouch with the “slot” in the black sample holder. The pouch will slide down into the sample holder until it reaches an internal “shelf”. This “shelf” ensures the sample is aligned in the impact zone of the tissueTUBE.

**NOTE:** A quick immersion in liquid nitrogen immediately before impact may aid pulverization of the sample.

### **Sample Pulverization**

**NOTE:** The apparatus has a safety interrupt on the lid. The system will not activate with the lid open.

Close the sample holder cover, select the desired impact level (1-6), and press the green “ACTIVATE” button.

**NOTE:** The system will not activate if the ACTIVATE is not green.

### **Pulverized Sample Transfer**

After impact, the pulverized sample in the tissueTUBE is ready for transfer into the attached culture tube.

1. Raise lid to remove the tissueTUBE-culture tube assembly from the CP02 and maintain the inverted position such that the tissueTUBE with the fractured sample is on the bottom.
2. Visually inspect to insure the tissue sample had been adequately impacted. If the sample was not completely pulverized, refreeze the tissueTUBE and then insert back into the CP02. Repeat the sample pulverization step.

**CAUTION:** If a second impact is required, be careful to inspect the pouch for punctures. If a puncture is noted, the pouch should not be impacted a second time.

**NOTE:** Some tissue may look compacted, rather than pulverized into a powder (e.g., fibrous muscle). However, the extra-cellular structure may have been fractured enough for efficient downstream extraction processes. While cold, pinch to dislodge flatten sample to enable transfer through tube opening. The energy of impact will melt water of the sample; however, since the sample is cold the water will quickly freeze (often in a flattened shape).

3. When the sample is adequately fractured, rotate (invert) the tissueTUBE-culture tube assembly such that the flexible pouch is on top. The sample is readily transferred to the culture tube.

**NOTE:** Prior to inversion, the processing tube should be chilled to prevent the cold contents from adhering to the inner walls of the tube as the pieces fall to the bottom.

4. A slight shake or flick motion to the pouch will aid transfer of tissue particles.

**NOTE:** A quick immersion in liquid nitrogen after impact pulverization may also aid transfer to the culture tube. When used correctly, most of the fractured sample is transferred to the processing tube.

5. Remove the tissueTUBE from the culture tube and discard it and its Plug

### **After Use**

If external cleaning of the CP02 is required, wash with a mild soap and water.

If cleaning of the impact area is required, unplug the unit. Rotate black lever 90°. (See Figure 2)

1. Remove the tissueTUBE sample holder carefully upward.
2. Grasp the sides of the clear cover with both hands and gently pull horizontally away from the apparatus. The clear cover may be washed with a mild soap and water. The impact area may be wiped with a dampened towel or cloth or tissue.
3. Reassemble and return the lever to its original position.

### **Quick Reference Procedure**

1. Load fresh tissue sample into tissueTUBE and freeze it immediately by placing in a cryo-environment (e.g. dry ice or mechanical freezer).
2. Prior to pulverization, remove the plug and affix a labeled processing tube (culture tube) to the tissueTUBE
3. Immerse pouch in dry ice (preferred) to maintain frozen state
4. Loosen ¼ turn if using a TT05, TT1 or TT2 sized tube.
5. Open the Pulverizer cover and quickly insert the pouch into the opening. The pouch will rest on a shelf while the glass tube is above. The glass tube acts as a handle. Close the cover.
6. Quickly depress the "ACTIVATE" button. Typically, the impact level is at setting 3 or 4.
7. Quickly open the cover, grab the glass tube, and remove the pulverized sample. Immerse in dry ice to cool transfer tube.
8. While holding the tissueTUBE, pinch flattened pieces (if any) to facilitate transfer through opening
9. Invert the tissueTUBE assembly to transfer pulverized sample to processing or storage tube
10. Remove tissueTUBE, affix cap for tube, and freeze until use
11. Discard the tissueTUBE and Plug

**Table 1**

## 6.0 Maintenance

### 6.1 Cleaning

The outside surfaces and switch overlay panel can be cleaned with a water-dampened cloth and mild detergent (automated, electric version). The surfaces can be cleaned with a mild detergent and disinfected if necessary by wiping with a cloth **dampened** with 70% alcohol or 10% bleach.

Excess liquid will harm the electronics and subsequent problems will not be covered under warranty. Do not use abrasives or solvents.

**CAUTION:** The electronics housing is not sealed. Excessive cleaning fluids could potentially leak into the system and cause an electrical shock hazard.

### 6.2 Storage Conditions

The CP02 components may be stored at room temperatures.

## 7.0 Specifications

<b>Model</b>	CP02
<b>Power Requirements:</b>	
<b>Check on label for voltage range</b>	100-120 VAC 300 VA, 50-60Hz for PN/500001 200-240 VAC 300 VA, 50-60Hz for PN/500000
<b>Dimensions</b>	23cm x 43cm x 27 cm
<b>Weight</b>	13 Kilograms
<b>Ambient Temperature Range:</b>	19°C to 25°C (66°F to 77°C)
<b>Ambient Humidity Range:</b>	30% to 70%

## 8.0 Service

There are no user-serviceable repairs.

**Decontamination** - Any machine or accessory containing blood and/or other biological or chemical deposits must be cleaned prior to shipment to the manufacturer/dealer for service. *This decontamination is required by Federal Law* (Title 48 and 49 of the Code of Federal Regulations) and in accordance with the Environmental Protection Agency's Regulations for Biohazard Waste Management. This decontamination cannot be performed by Covaris personnel.

## 9.0 Troubleshooting Guide

The key to successful operation of the cryoPREP system is to insure the temperature of the samples are maintained at a subzero level (preferably, less than minus 60°C). This is facilitated by efficient handling and processing of the samples.

<b>Problem</b>	<b>Possible Cause</b>	<b>Recommendation</b>
Sample is partially pulverized	It is important that sample is in the middle of the tissueTUBE. Otherwise, the samples may be out of the impact area and will not be pulverized.	The best way to ensure this is to inspect the tissueTUBE after the sample is loaded to be certain the sample is in the middle of the tube
Small samples are pulverized to very fine powder	Small masses and tissues without extensive extracellular matrix, such as small amounts of brain specimens, may fracture too finely	Choose a lower impact level.
Sample is flattened after impact	Some samples, depending on a high degree of fibrous tissue, may not pulverize to a fine powder. The appearance may be “flattened”; however, the extra-cellular matrix of the tissue has been disrupted or fractured. Tissue that has a flattened appearance may be appropriate for downstream homogenization or extraction as the matrix is disrupted.  Samples that are highly fibrous, such as muscle tissue pulverize better at colder temperatures.	Initial sample was not cold enough or pulverized sample thawed prior to transfer. It is imperative to work quickly as the thermal imbalance is over 100 degree C.  It is recommended the fibrous samples are immersed into liquid nitrogen for a couple of seconds prior to insertion into the CP02 and are then quickly impact pulverized.
Portion of pulverized sample is retained on the inside of tissueTUBE after transfer	In the tissueTUBE, the Sample temperature became too high. This may occur as the sample temperature is too warm or the time to transfer after pulverization is too slow	Immerse the pouch portion of the tissueTUBE into dry ice or liquid nitrogen for a couple of seconds before and/or after pulverization.
Power is on, but Activate light is not lit	<ul style="list-style-type: none"> <li>• One of the safety interrupts has not been reset</li> <li>• Cover is not on securely</li> <li>• Lid is open</li> </ul>	<ul style="list-style-type: none"> <li>• Lift and reclose lid</li> <li>• Reposition the cover</li> <li>• Close the lid</li> </ul>
During transfer, the sample adheres to the walls of the process tube	Process tube temperature is too warm as the powdered material thaws and adheres to inner surfaces	Ensure the process tube (e.g., Fisher 16mm x 100mm) is at a cryogenic temperature. Typically, this takes a minute on dry ice to cool the glass tube.
tissueTUBE does not fit into Tube Holder	The wrong tissueTUBE holder is inserted. For example, TT1 or TT1-XT will use the holder labeled TT1 and TT2 will use the holder labeled TT2.	

## Appendix A: References

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Blood borne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A3 Clinical Laboratory Safety; Approved Guideline - Third Edition, published 2012, ISBN 1-56238-797-9
- Clinical and Laboratory Standards Institute (CLSI), M29-A4 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Fourth Edition, published 2014, ISBN 1-56238-961-0
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- Botwell, D. and Sambrook, J. (2003) DNA Microarrays, A Molecular Cloning Manual, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, NY.

## Appendix B: tissueTUBE Sizes

Sample mass	tissueTUBE	tissueTUBE Plug	tissueTUBE Insertion Tool	tissueTUBE Holder
< 50 mg (*)	TT05M (PN 520139)	TT05M-P (PN 520141)	TT05 (PN 500231)	TT1 (PN 500095)
< 50 mg of hard tissue (heart, muscle, kidney) (*)	TT05M-XT (PN 520140)	TT05M-P (PN 520141)	TT05 (PN 500231)	TT1 (PN 500095)
(*) Legacy TT05 (PN 520071) and TT05XT (520072) are also available for this application, please contact <a href="mailto:applicationsupport@covaris.com">applicationsupport@covaris.com</a>				
< 500 mg	TT1 (PN 520001)	TT1-P (PN 520006)	TT1 (PN 500159)	TT1 (PN 500095)
< 500 mg hard tissue (bone, seeds, tablets, plant material)	TT1-XT (PN 520007)	TT1-P (PN 520006)	TT1 (PN 500159)	TT1 (PN 500095)
500 mg - 2 g	TT2 (PN 520021)	TT2-P (PN 520023)	N/A	TT2 (PN 500096)

See Appendix C for holders and racks for the various Covaris Focused-ultrasonicators

## Appendix C: Ordering Information

model number	part number	description
CP02 (120V)	500001	Automated system with variable force electronically controlled cryogenic sample pulverization. Includes Accessory Kit
CP02 (220V)	500000	Automated system with variable force electronically controlled cryogenic sample pulverization. Includes Accessory Kit
Kits		
TT1 KIT	500097	Accessory Kit included with automated cryoPREP System (CP02): (1) TT1 Tube Holder, (25)TT1, (25) TT1-P, (25) 16 x 100 mm tubes&caps
TT2 KIT	500140	Accessory Kit included with automated cryoPREP System (CP02): (1) TT2 Tube Holder, (25)TT2, (25) TT2-P, (25) 20 x 125 mm tubes&caps
<b>tissueTUBEs &amp; Accessories</b>		
TT05M	520139	tissueTUBE for sample collection, storage, pulverization and transfer. For samples of mass <50mg. For use with milliTUBE transfer vessels
TT05M-XT	520140	tissueTUBE EXTRA THICK for sample collection, storage, pulverization, and transfer. For samples of mass <50mg. For use with milliTUBE transfer vessels.
TT05M-Plug	520141	Plugs for use with the TT05M and TT05M-XT. Polypropylene with silicon O-ring for cryogenic storage.
Cold Station TT05	500249	Cold Station for TT05M, and TT05M-XTwith appropriate 12x24 mm tube and 1ml and 2ml milliTUBE transfer vessels.
TT05 Insertion Tool	500231	Insertion tool for TT05M, and TT05M-XT tissueTUBEs
TT1	520001	tissueTUBE for sample collection, storage, pulverization, and transfer. For samples of mass <1gm. For use with the CP-01 and CP-02.
TT1-XT	520007	tissueTUBE EXTRA THICK for sample collection, storage, pulverization, and transfer. For samples of mass <1gm. For use with the CP-01 and02
TT1-P	520006	Plugs for use with the TT1 and TT1-XT. Polypropylene with silicon O-ring for cryogenic storage.
TT1ADP12	520062	tissueTUBE (TT1) threaded adapter for 520056 (12 x 24mm tubes).
TT1ADP13	520017	tissueTUBE (TT1) adapter for 13 x 65 mm tubes. 25/bag
TT1 milliTUBE Adaptor	520142	tissueTUBE (TT1) adapter for 1ml and 2ml milliTUBEs. 25/bag
TT1 Insertion Tool	500159	Insertion tool for the 520017 adapter for use with either 14x19 mm, 12x24 or 12x12 mm tubes.
TT1-R	500041	Sample collection rack for 8 TT1. Designed for immersion into liquid nitrogen.
TT1 Holder	500095	cryoPREP tissueTUBE holder for TT1 TT1-XT and TT05, TT05-XT, TT05M, and TT05M-XT
TT2	520021	tissueTUBE for sample collection, storage, pulverization, and transfer. For samples of mass <2 gm. For use with the CP-01 and CP02.
TT2-P	520023	Plugs for use with the TT2. Polypropylene with silicon O-ring for cryogenic storage.
TT2 Holder	500096	cryoPREP tissueTUBE holder for TT2
AFA Transfer Tubes, Holders, & Racks		
TC13	520010	Transfer Tube for use with TT1. 13 x 65 mm tubes (borosilicate) and caps (polypropylene). <b>Requires use of TT1ADP13 (520017)</b>
TC16	520011	Transfer Tube for use with TT1. 16 x 100 mm tubes (borosilicate) and caps (polypropylene) 500 each

TC16	520191	Transfer Tube for use with TT1. 16 x 100 mm tubes (borosilicate) and caps (polypropylene) 25 each
TC20	520012	Transfer Tube for use with TT2. 20 x 125 mm tubes (borosilicate) and caps (polypropylene) 500 each
TC20	520192	Transfer Tube for use with TT2. 20 x 125 mm tubes (borosilicate) and caps (polypropylene) 25 each
milliTUBE 1ml AFA Fiber	520135	Transfer Tube for use with TT05M and TT05M-XT. 12x12 tube w/AFA fiber and PTFE seal cap (vol. 1ml), 150 PIP limit. 24 each.
milliTUBE 2ml AFA Fiber	520186	Transfer Tube for use with TT05M and TT05M-XT. 12x24 tube w/AFA fiber and PTFE seal cap (vol. 1ml), 150 PIP limit. 24 each.
TR-12-16	500031	Rack (12 place) for 16 x 100 mm tubes. <b>E220 only.</b>
TR-12-20	500032	Rack (12 place) for 20 x 125 mm tubes. <b>E220 only.</b>
Rack, milliTUBE 1ml	500368	Rack (24 place) for milliTUBE 1ml. <b>E220 and LE220 only.</b>
Rack, milliTUBE 2ml	500376	Rack (24 place) for milliTUBE 2ml. <b>E220 and LE220 only.</b>
Rack, milliTUBE	500431	Rack (4 place) for milliTUBE 1ml. <b>E220 evolution only.</b>
THQ16	500012	Holder for (1) 16 x 100 mm tube for the <b>S220</b> .
THQ20	500051	Holder for (1) 20 x 125 mm tube for the <b>S220</b> .
Holder, 1ml milliTUBE	500371	Holder for (1) 1ml milliTUBE for the <b>S220</b>
Holder, 2ml milliTUBE	500375	Holder for (1) 2ml milliTUBE for the <b>S220</b>
Rack, milliTUBE	500520	Rack (4 place) for milliTUBE 1ml. <b>ME220 only.</b>
XTU Holder	500414	Universal Holder for <b>M220 only</b> . Used with tube specific inserts; see below.
Insert, milliTUBE	500422	milliTUBE insert for M220 XTU holder. <b>M220 only</b>

*\*Requires use of TT1ADP13 (520017)*

