



For research use only

CP01 CryoPREP® Manual Dry Pulverizer

USER MANUAL

Instrument developed for tissue sample stabilization, pulverization, and storage.

Product Name: CP01 cryoPREP Manual Dry Pulverizer

Product Number: CP-01



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 Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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Not for use in diagnostic procedures

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Warnings

For safety of operating personnel:

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:

The black Cradle and tissueTUBE™ adapters should not be stored in cold environments. Doing so may make them brittle and shatter upon impact.

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 60 days 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, U.S.A. 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.

Warranty Exceptions

This warranty is void if failure of the 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.

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

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

- Telephone during the hours of 9:00am to 5:00pm, Monday through Friday, EST. +1 781 932 3959.
- E-mail queries to applicationsupport@covaris.com

An Operator's Manual is provided with the equipment. This manual includes sections on the operating instructions, maintenance guidelines, and troubleshooting.

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1.0 INTRODUCTION

1.1 Overview of the Manual

This manual contains operation and service instructions for the Covaris CP01 cryoPREP Manual Dry Pulverizer. 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 Application 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 readily transfer the contents to a downstream processing tube at cryogenic temperatures.

The patent pending cryoPREP system is based on the effective pulverization of biological samples in a cryogenic 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 (TT1& TT05M), is a single-use design that eliminates cross-contamination. The tissueTUBEs are constructed of engineering polymers (for example, Teflon® / Kapton® composite films). The tissueTUBE is used with a hammer / anvil means to generate an impact force. These components when used together comprise the cryoPREP system.

Covaris' single-use, closed-tube cryogenic 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, 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 CP01 cryoPREP System Components

The cryoPREP system is composed of the following components:

- **Removable Pulverizer** – A stainless steel assembly that provides the hammer and anvil pulverizing action to the sample.
- **Hammer** – An 18 ounce hammer is used to deliver the blow that will fracture the sample.
- **Cradle** – Aligns the Removable Pulverizer with the tissueTUBE Adapters.
- **tissueTUBE Adapters** – There are separate adapters for the TT1 and TT05M. Their function is to align a tissueTUBE with the Removable Pulverizer. They easily slide in and out of the Cradle to change between the two.



Figure 1

1.4 Overview of CP01 Compatible Consumables

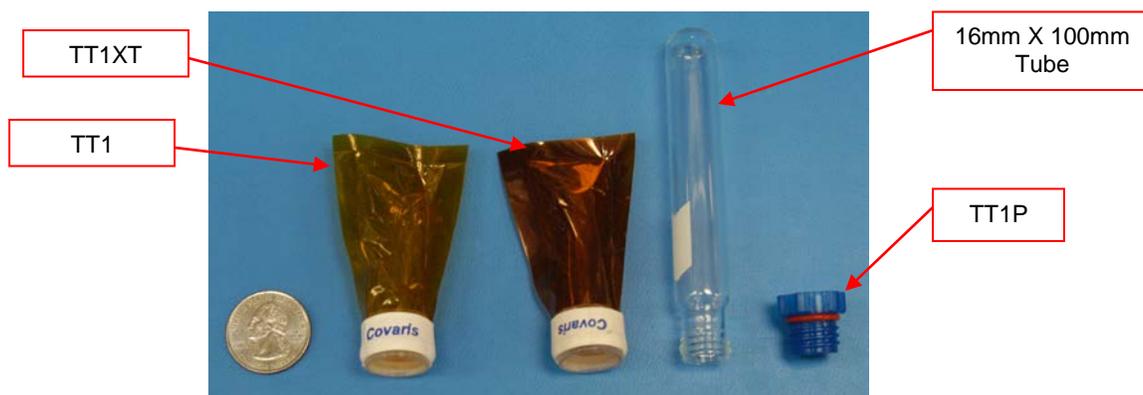


Figure 2

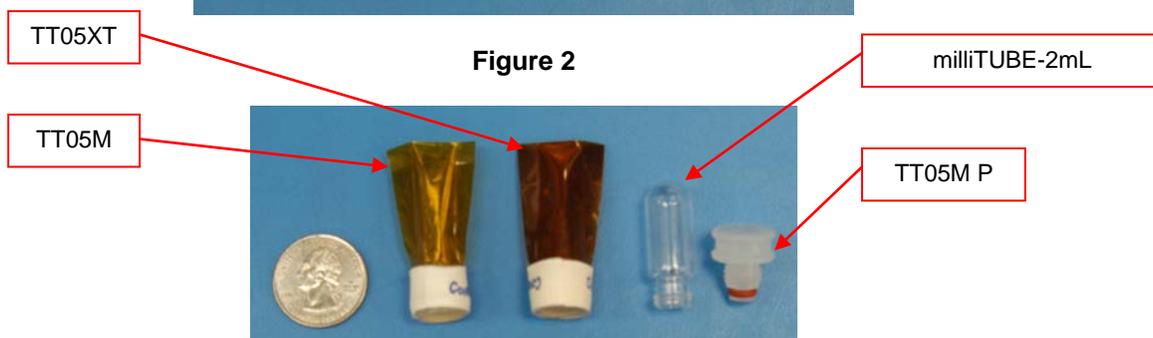


Figure 3

- **TT05M** - A single-use, sample pulverizing tube designed with a threaded hub for rapid transfer of contents into a milliTUBE. The TT05M may be directly immersed in liquid nitrogen or stored at -80C. (PN: 520139)
- **TT05M XT** – A thicker variation of the TT05M for tougher tissue samples. (PN: 520140)
- **TT05M P** – A plug with an O-ring that facilitates long-term cryogenic storage for samples in a TT05M or TT05M XT. (PN: 520141)
- **milliTUBE 1 mL** – A 1 mL transfer tube that connects directly to the TT05M and TT05M XT tissueTUBE. (PN: 520128)
- **milliTUBE 2 mL** – A 2 mL transfer tube that connects directly to the TT05M and TT05M XT tissueTUBE. (PN: 520132)
- **TT1** - A single-use tube designed with a threaded hub for rapid transfer of contents to 16mm x 100mm screw-thread tubes. The TT1 may be directly immersed in liquid nitrogen or stored at -80°C. (PN: 520001)
- **TT1XT** – A thicker variation of the TT1 meant for tougher tissue samples. (PN: 520007)
- **16mm X 100mm Tube** – A borosilicate glass, round bottom tube with a writing patch allows further sample processing and storage. It comes with a polypropylene screw cap. (PN: 520011)

- **TT1P** – A plug with an O-ring that facilitates long term cryogenic storage for samples in a TT1 or TT1XT. (PN: 520006)

1.5 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 TT1 is designed for use with biological tissue samples of up to 1 g. Tissue masses greater than 1 g will not be completely in the contact area for the pulverization and consequently, the pulverization of samples greater than 1 g will be incomplete.

The TT05M is designed for use with biological tissue samples of up to 0.05 g. Using tissue masses greater than 0.05 g may cause the seam of the bag to fail.

The TT1 and TT05M are designed for biological samples with a three-dimensional volume. For example, some standard mammalian tissues which are appropriate for use with the TT1 and TT05:

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

Samples such as cell culture pellets are not appropriate.

The TT1XT and TT05M XT are a special type of tissueTUBE, designed to withstand greater stress at cryogenic temperatures for harder tissues. The XTs are constructed with a thicker pouch material. Their samples are, but not limited to:

- Bone
- Seed
- Plant stem material

The XTs may also be used with softer tissues, although most tissues are appropriate for the standard TT1 and TT05M tissueTUBEs.

2.0 Unpacking and Installation

2.1 Check for damage

The cryoPREP system and its accessories are delivered in one box. If any of the parts have suffered any damage in transport, please inform your carrier immediately. If possible, save the shipping box with all of its components in the event that the apparatus needs to be returned for service.

2.2 Installing the CP01 cryoPREP Instrument

Place the cryoPREP system on any solid surface acceptable for laboratory instrumentation and capable of withstanding the force of the hammer. Do not use the system near other equipment or materials sensitive to the bench-top vibrations caused by the hammer impact.

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 management or supervisory and safety personnel to ensure adequate training of operators as to the safe and effective use of the apparatus.

3.2 Hazards

The CP01 cryoPREP system has a zone in the Removable Pulverizer that presents a pinch hazard. It is the operator's responsibility to avoid this pinch hazard as well as the strike zone of the hammer when using the CP01.

3.3 Safety Information

The CP01 cryoPREP 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.

Gloves are required, and cryogenic gloves are highly recommended for handling the pre-chilled Pulverizer Assembly, TT1 and TT05M.

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

As the Removable Pulverizer is subjected to cryogenic temperatures, when the relative humidity is high there will be condensation frost on the apparatus. The condensation will not adversely affect the performance as long as the cylinder is able to move freely. High impact forces will dislodge ice / frost.

The TT1 and TT05M are subjected to cryogenic temperatures. When the relative humidity is high, there will be condensation frost on the screw threads. As a result, this may cause resistance when trying to unscrew a tube or plug from the tissueTUBE.

4.0 Protocol

4.1 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 cryoPREP system requires an external chilling source to maintain the Removable Pulverizer and the samples at cryogenic temperatures. The following may be used to condition the samples prior to sample pulverization:
 - Dry ice
 - Liquid nitrogen

- Mechanical Freezer (-80°C)

For example, samples may be stored in the TT1 in a freezer, the TT1 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 the 16mm x 100mm as the “transfer tube” for the TT1. The milliTUBEs act as the “transfer tube” for the TT05M tissueTUBE.
- **Labeling Strategy** – The 16mm x 100mm 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 TT1 may also be written on with a permanent marker pen. Alternatively, a label may be affixed to the white portion of the TT1.
- **Freezing Strategy** – The tissueTUBE may be immersed directly into liquid nitrogen, therefore, a vessel to contain the fluid is required. In addition, if multiple samples are taken in a short time period, it may be more convenient to have several tissueTUBEs inverted in a rack holder and partially immersed in liquid nitrogen prior to fresh tissue sample loading.

The use of dry ice will also require the use of a tray or vessel to contain the material. The tissueTUBE may be directly laid horizontally on the dry ice.

- **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 TT1 or TT05M) 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, loading of the tissueTUBE and insertion into a mechanical freezer may be adequate.

NOTE: After the sample has been pulverized, it is possible to affix the plug and use the TT1 or TT05M as a storage vessel. Aliquots of the 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.

4.2 Procedure for preparing pulverized tissue

Sample Loading into the tissueTUBE (TT1 & TT05M)

The TT1 is designed for cryogenic processing of samples less than 1 g and the TT05M samples less than .05 g. There are two parts: a threaded hub to affix to the culture tube and a flexible pouch to hold the sample. The freshly procured samples are to be inserted through the threaded opening, per the suggested procedure below:

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 near the bottom 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 into a tissueTUBE, connect a compatible transfer tube.
4. Immerse the flexible pouch portion of the tissueTUBE into a cryogenic environment (e.g., dry ice or liquid nitrogen). The sample will be frozen within 30-90 seconds.
5. After the sample is frozen, it may be stored at -80C or may be immediately used for tissue pulverization.

Sample Loading into the CP01 cryoPREP Manual Dry Pulverizer

1. Remove a previously chilled Removable Pulverizer from a subzero environment. Make sure that your hand is protected from frostbite by using an appropriate glove.



Figure 4

2. Place the Removable Pulverizer into the Cradle with the correct tissueTUBE Adapter.
3. With gloved hands, raise the cylinder of the Removable Pulverizer and insert the chilled tissueTUBE into the Adapter until it bottoms out. For best pulverization results, dip the tissueTUBE in Liquid Nitrogen immediately before loading it into the Adapter.

CAUTION: The tissueTUBE Assembly should be vented before striking it. This requires the operator to unscrew the tube by a quarter turn. If the operator is using a TT1P or TT05P, unscrew the plug until the O-ring seal is broken and the bag offers no resistance when squeezed. **Failure to vent may cushion the impact or burst the bag.**

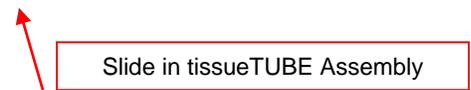


Figure 5

4. Release the cylinder. Strike the top of the cylinder with the Hammer to pulverize the sample.



Figure 6

Pulverized Sample Transfer

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

1. Raise the cylinder with a gloved hand and slide the tissueTUBE free of the Adapter.
2. Visually inspect to ensure the tissue sample has been adequately impacted. If the sample was not completely pulverized, the sample may need to be repositioned in the tissueTUBE and struck again.

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. Before a second impact, the sample must be flash frozen in liquid nitrogen for an additional 30-90 seconds.

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 flattened 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 pulverized, rotate the tissueTUBE assembly so that the bag is on top. As the inner surface of the bag is coated with Teflon, 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.

5.0 Maintenance

5.1 Cleaning

The CP01 may be rinsed with soap and water. Do not use abrasives or solvents.

5.2 Storage Conditions

The CP01 components should be stored at room temperatures. Only the Removable Pulverizer and tissueTUBE should experience subzero temperature.

5.3 Technical Assistance

Contact Covaris at applicationsupport@covaris.com or +1 781 932 3959 (during hours of 9:00am to 5:00pm, Monday through Friday, EST)

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.

6.0 Troubleshooting Guide

The key to successful operation of the cryoPREP system is to ensure 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.

Problem	Possible Cause	Recommendation
Sample is partially pulverized	It is important that sample is in the bottom area of the TT1. Samples that are not at the end of the TT1 may be out of the impact area and will not be pulverized.	The best way to ensure this is to inspect the TT1 after the sample is loaded to be certain the sample is in the bottom 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	Reduce impact force with the hammer
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/fractured. Tissue that has a flattened appearance may be appropriate for downstream homogenization or extraction as the matrix is disrupted.	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°C.
	Samples that are highly fibrous, such as muscle tissue pulverize better at colder temperatures.	Immerse the pouch portion of the tissueTUBE into liquid nitrogen for a couple of seconds before pulverization.
Portion of pulverized sample remains on 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 liquid nitrogen for a couple of seconds before and/or after pulverization.
During transfer, the sample adheres to the walls of the process tube	Process tube temperature is too warm as the powered material thaws and adheres to inner surfaces	Ensure the process tube (e.g., Fisher 16mm x 100m) is at a cryogenic temperature. Typically, this takes a minute on dry ice to cool the glass tube.