PROTOCOL

Covaris

Tissue processing with the CP01 cryoPREP®
Manual Dry Pulverizer

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INTENDED USE

The CP01 cryoPREP Manual Dry Pulverizer is intended for use in research applications (RUO). This product is not intended for the diagnosis, prevention, or treatment of disease.

INTRODUCTION

The cryoPREP Dry Impactors have been designed to integrate the tissue processing workflow from flash freezing to biomolecule extraction using the Adaptive Focused Acoustics® (AFA®) technology.

The dry pulverization process disrupts the extra-cellular tissue matrix and increases the tissue surface area, thus improving the extraction efficiency of biomolecules. This increased surface area reduces the distance a stabilizing extraction buffer need to diffuse during subsequent lysis steps.

As the Covaris lysis process is independent of buffer choice, the most appropriate buffer for the downstream analytical method is selected. This tool can be used to process tissues for the isolation of DNA, RNA, proteins, metabolites, and other target biomolecules.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010458	A	06/18	As released
010458	B	10/18	Update product name and add materials required

DESCRIPTION OF THE WORKFLOW

The biomolecule extraction workflow is a two steps process:

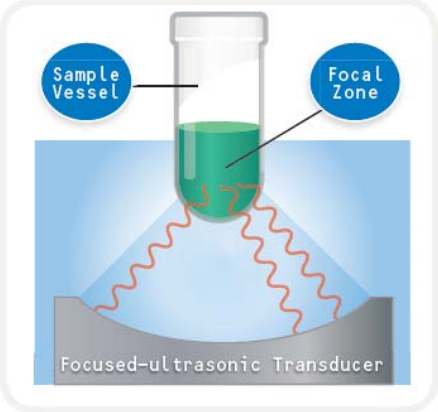
1. Tissue cryo-pulverization with the CP01 cryoPREP Manual Dry Pulverizer
2. Active homogenization with the Covaris Adaptive Focused Acoustic (AFA) technology

Dry tissue cryo-pulverization

The tissue should be loaded into the center of the tissueTUBE followed by treatment in liquid nitrogen (LN_2) to flash freeze the tissue. The flash frozen tissue can then be processed on the Covaris CP01 cryoPREP Manual Dry Pulverizer to disrupt the extra-cellular tissue matrix. The pulverized tissue material is then transferred into a Covaris AFA tube for homogenization.

Enhanced biomolecule extraction using AFA

Extraction of the target biomolecules is significantly enhanced when using the Covaris AFA technology paired with an appropriate lysis buffer. Different instruments are available depending of tissue mass and desired throughput. Please refer to Covaris website for details on instruments and capacities.



Materials Required

The CP01 cryoPREP Manual Dry Pulverizer can be used to process tissue samples within a mass range of 15 mg to 1 g.

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 -80ºC. (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)

PROTOCOL

Sample Loading into the tissueTUBE

1. While holding the tissueTUBE (TT05M or TT1 tissueTUBE), insert the sample specimen using forceps or tweezers through the office of the bag.

2. Ensure the tissue sample is inserted and placed in the middle of the tissueTUBE by pitching the bottom of the bag.

> **Note1:** The TT1 and TT05M are designed for biological samples, such as liver, kidney, skeletal muscle, cardiac, lung, brain, and adipose tissue.
> **Note2:** The TT1-XT and TT05M-XT are designed to withstand greater stress at cryogenic temperatures and recommended for use with harder tissue types, such as bone.

3. After loading the sample into the tissueTUBE, attach the appropriate transfer tube and place on dry ice for at least 2 minutes.

4. Remove the tissue tube-holder assembly from dry ice and submerge the bottom 2/3 of the tissue tube into liquid nitrogen (LN_2) for 30-90 seconds to flash freeze the sample.

5. Immediately load the flash frozen tissue sample into the holder.

Sample Loading into the CP01 cryoPREP Manual Dry Pulverizer

1. Using protective gloves, remove the pre-chilled CP01 cryoPREP Manual Dry Pulverizer from the freezer (-80C).

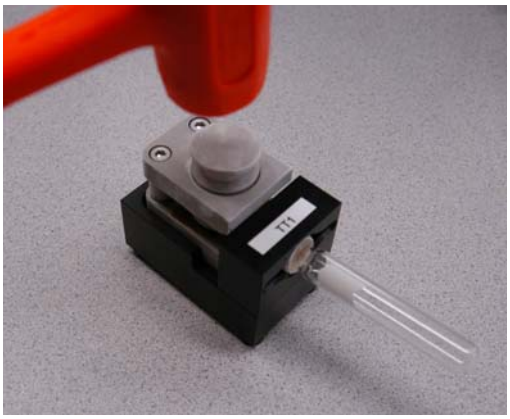
2. Place the pre-chilled portion of the CP01 cryoPREP Manual Dry Pulverizer into the holder (black piece) with the correct tissue tube adapter.

3. With gloved hands, raise the cylinder on the CP01 cryoPREP Manual Dry Pulverizer and insert the chilled tissueTUBE into the adapter. For best pulverization results, dip the

tissueTUBE into liquid nitrogen (LN_2) immediately before loading for 30-90 seconds depending on the tissue mass.

Note: The tissueTUBE assembly must be vented before striking it to prevent the tissueTUBE from breaking. To do this, unscrew the transfer tube by a quarter of a turn prior to inserting into the holder.

4. Release the cylinder. Strike the top of the cylinder with the Covaris impactor as demonstrated below to pulverize the sample.



5. After impact, the pulverized sample can be transferred to the transfer tube. To do this, raise the cylinder with a gloved hand and remove the tissueTUBE.

6. 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.

Note 1: If a second impact is required, inspect the pouch to ensure no punctures are present and flash freeze in liquid nitrogen before pulverizing the sample for a second time. If a puncture is noted, the pouch should not be impacted a second time.
Note 2: A quick immersion in liquid nitrogen (LN_2) after pulverization may also aid in the transfer process.
Note 3: Prior to inversion, the tissueTUBE should be chilled to prevent the cold contents from adhering to the inner walls of the tube as the pieces fall to the bottom.
Note 4: A slight shake or flick motion to the pouch will aid transfer of tissue particles.

SUPPLEMENTAL MATERIAL

Appendix A: Troubleshooting Guide

Problem	Possible Cause	Recommendation
Sample is partially pulverized	It is important for the sample to be in the bottom area of the tissueTUBE. Samples that are not at the end of the tissueTUBE may be out of the impact area and will not be pulverized effectively.	The best way to ensure this is to inspect the tissueTUBE after the sample is loaded to be certain the sample is in the bottom of the tissueTUBE.
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 100C.
Portion of pulverized sample remains on inside of tissueTUBE after transfer	This may occur if 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.

Technical Support

If you need assistance at any time, please contact Covaris using the information below.

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