

Tissue Processing with the CP01 cryoPREP® Manual Dry Pulverizer

Developed for Tissue Sample Stabilization, Pulverization, and Storage

Table of Contents

Intended Use.....	3
Introduction.....	3
CP01 cryoPREP Manual Dry Pulverizer	4
Biomolecule Extraction Workflow	4
Dry Tissue Cryo-pulverization.....	4
Enhanced Biomolecule Extraction using AFA®	4
Laboratory Equipment, Chemicals, and Consumables Supplied by User.....	5
Protocol.....	6
Sample Loading into the tissueTUBE	6
Sample Loading into the CP01 cryoPREP Manual Dry Pulverizer.....	6
Appendix A: Troubleshooting Guide	8
Technical Support and Assistance	9
Revision History.....	9

General Information

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 government and clinical standards, or their equivalent in the country of use. For more details, visit www.covaris.com/universal-precautions.

Trademarks

Patented or patent pending and registered or registration-pending trademarks of Covaris are protected. Registered names and trademarks used in this document, even when not specifically marked as such, are not to be considered unprotected by law. WCS and AFA are either registered trademarks or trademarks of Covaris, LLC in the United States and/or other countries. Information subject to change without notice. For Research Use Only (RUO). Not for use in Diagnostic Procedures. Product covered by Patent No. US6, 719,449, US6, 948,843, and other pending applications.

2022© Covaris, LLC. All Rights Reserved. 14 Gill St., Woburn, MA 01801, USA.

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 Pulverizers 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 needs 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.

CP01 cryoPREP Manual Dry Pulverizer

Biomolecule Extraction Workflow

The workflow is a two-step process:

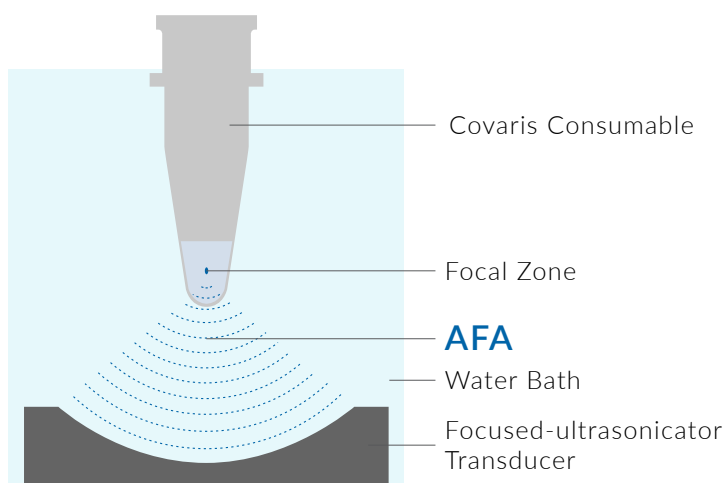
1. Tissue cryo-pulverization with the CP01 cryoPREP Manual Dry Pulverizer
2. Active homogenization with the Covaris Adaptive Focused Acoustics (AFA) technology

Dry Tissue Cryo-pulverization

The tissue should be loaded into the center of the tissueTUBE followed by treatment in liquid nitrogen (LN2) 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 on tissue mass and desired throughput. Please refer to the Covaris website (www.covaris.com) for details on instruments and capacities.



Laboratory Equipment, Chemicals, and Consumables Supplied by User

Required Laboratory Equipment and Accessories

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

Product Name	Product #	Product Description
tissueTUBE TT05M	520139	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.
tissueTUBE Extra Thick TT05M XT	520140	A thicker variation of the TT05M for tougher tissue samples.
tissueTUBE TT05M Plug	520141	O-ring plug that facilitates long-term cryogenic storage for samples in a TT05M or TT05M XT.
milliTUBE 1 mL	520128	1 mL transfer tube that connects directly to the TT05M and TT05M XT or, with the use of TT1ADPM, TT1 and TT1 XT tissueTUBES.
milliTUBE 2 mL	520132	2 mL transfer tube that connects directly to the TT05M and TT05M XT or, with the use of TT1ADPM, TT1 and TT1 XT tissueTUBES.
tissueTUBE TT1	520001	Single-use tube designed with a threaded hub for rapid transfer of contents to a transfer tube. The TT1 may be directly immersed in liquid nitrogen or stored at - 80 °C.
tissueTUBE TT1 Extra Thick	520007	A thicker variation of the TT1 meant for tougher tissue samples.
TC16 Tube	520011	A 16x100mm, round bottom, transfer tube made of borosilicate glass, with a writing patch that allows for further sample processing and/or storage. It includes a polypropylene screw cap.
tissueTUBE TT1 Plug	520006	O-ring plug that facilitates long term cryogenic storage for samples in a TT1 or TT1XT.
TT1ADPM	520142	tissueTUBE adaptor for use with 1 or 2 mL milliTUBE(s).

Protocol

Sample Loading into the tissueTUBE

1. While holding the tissueTUBE (TT05M or TT1 tissueTUBE), using forceps or tweezers, insert the sample specimen in the bag.
2. Ensure the tissue sample is inserted and placed in the middle of the tissueTUBE by pinching the edges of the bottom of the bag.

NOTE: The TT1 and TT05M are designed for biological samples, such as liver, kidney, skeletal muscle, cardiac, lung, brain, and adipose tissue.

NOTE: 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 tissueTUBE transfer tube assembly from dry ice and submerge the bottom 2/3 of the tissueTUBE into liquid nitrogen (LN2) 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 (- 80 °C).



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 (LN2) immediately before loading for 30 - 90 seconds.

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, raise the cylinder with a gloved hand and remove the tissueTUBE.
6. Visually inspect to ensure that 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: 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, transfer the sample to a new tissueTUBE for a second impact, and discard the punctured pouch.

7. The adequately pulverized sample can then be transferred to the transfer tube. To do this, remove the tissueTUBE from the cylinder and orient it in a vertical direction with the tissueTUBE above the transfer tube.

NOTE: A quick immersion in liquid nitrogen (LN2) after pulverization may also aid in the transfer process.

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

Appendix

Appendix A: Troubleshooting Guide

Symptom	Possible Causes	Solution
Sample is partially pulverized	It is important for the sample to be positioned towards the middle area of the tissueTUBE. Samples that are 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 middle 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 100 °C.
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

Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone:
 - US: +1 781.932.3959, during the hours of 9:00 a.m. to 4:00 p.m. (EST), Monday through Friday
 - EU: +44 (0)845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. (GMT), Monday through Friday
- E-mail instrumentation queries to techsupport@covaris.com and application queries to applicationsupport@covaris.com

Revision History

Document Part #	Revision	Date	Description of change
010458	A	06/2018	Initial release of document
010458	B	10/2018	Updated product name and additional materials required
010458	C	11/2022	Update formatting and trademarks