

PRODUCT INSERT: tissueTUBEs and Plugs (TT1, TT1-XT and TT1-P)**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

**CRYOGENIC HAZARD**

The cryoPREP system requires the use of low temperature materials and equipment, including -80°C freezers, dry ice, and/or liquid nitrogen. Personnel must be trained to safely handle these materials and associated equipment.

Storage Conditions Prior to Use

TT1 and TT1-XT tissueTUBEs and tissueTUBE Plugs (TT1-P) may be stored at room temperature until employed and are stable for at least one year under these conditions. There is an expiration date on all packaging.

Limitations on in vitro Usage

TT1, TT1-XT, and TT1-P are developed, designed, and sold for research use only. They are not to be used for human diagnostic purposes or treatment unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use.

Product Warranty Guarantee

Covaris guarantees the performance of all products when used in accordance with our written instruction, under normal operating conditions, and during the expiration period. The user must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any other reason than misuse, Covaris will replace it free of charge. We reserve the right to change, alter, or modify any product to enhance its performance or design. If a product does not meet your expectations, please contact Covaris Technical Assistance.

Technical Assistance

On-going assistance with the operation or application of any of our products is provided via:

- Telephone during the hours of 9AM to 5PM, Monday through Friday, (GMT-05:00) Eastern Time (US & Canada) +1 781 932 3959
- E-mail queries to applicationsupport@covaris.com

INSTRUCTIONS FOR USE *(Please refer to the cryoPREP User Manual for further instructions on use of this instrument)*

TissueTUBEs will be referred to as TT1 for the remainder of this insert.

Purpose

The cryoPREP system employs a programmable impact hammer to pulverize cryogenically frozen tissue samples, employing the brittleness of frozen biological samples for reproducible pulverization. The process increases sample surface area and breaks extra-cellular matrices. The TT1 tube and plug are designed to allow easy transfer of the pulverized material to a 16mm diameter tissue vial. During subsequent treatment in Covaris AFA instruments such as the S-Series and E-Series, homogenization times are reduced and the extraction efficiency of target bio-molecules improved, enabling larger sample size and higher throughput.

Equipment Notes

The TT1 tissueTUBE is designed to be used with the Covaris cryoPREP CP02 instrument. The CP02 is available in either a 100-120 volt model (PN 500001) for use in the Americas or Japan or in a 200-240 volt model (PN 500000) for use in Europe, Asia and Australia.

The CP02 must be equipped with a TT1 Holder (Covaris PN 500095) to accept TT1 sample tubes.

The TT1 is designed for use with biological tissue samples of 100 to 1000 milligram mass. Typical samples range from 250mg to 500mg. Appropriate tissues to use with the TT1 include liver, kidney, skeletal muscle, heart, dermal, lung, brain, and adipose.

The TT1-XT is a thicker walled tube designed to withstand greater stress. The TT1-XT is recommended for hard tissue such as: bone, seeds, plant material, and tablets. Cell culture pellets are not appropriate for use in either version of TT1 tube. Do not exceed 1000mg of mass.

The TT1 employs a 15-415 threaded top to mate with a standard 16x100 mm culture tube. Covaris recommends a disposable, borosilicate, round bottom glass tube with polypropylene cap (Covaris PN 520011, Fisher Scientific PN 14-962-26F or VWR PN 60825-837). For applications that employ other tubes sizes, please contact Covaris.

Sample Processing Instructions

- Place TT1 Prep Station into ice tray and add dry ice.
- Previously labeled culture tube can be pre-chilled by placing them into the second position in the TT1 Prep Station shown in Figure 1.
- Insert the sample specimen through the top opening of the TT1 as illustrated, using forceps or tweezers. Place the sample in the center of the flexible pouch, away from the edges.
- After the sample is loaded, seal the TT1 by screwing a TT1-P plug or appropriate transfer tubes tube into the top of the TT1. If multiple samples are being processed, they should be identified by the tube label or a tag on the TT1 cap. Do not label the flexible pouch.
- While holding either the plug or the culture tube, freeze the sample by immersing the flexible pouch into a cryogenic environment (e.g., dry ice or liquid nitrogen). If using the TT1 Prep Station; freeze the sample by inserting the flexible pouch into the first position in the TT1 Prep Station as shown in Figure 1. If using liquid nitrogen, dip only the sample pouch and avoid dipping the cap or glass tube. The sample may now be stored at -80°C or in dry ice prior to pulverization.
- When sample is frozen replace the plug with a pre-chilled culture tube. The sample can be placed in the third position in the TT1 Prep Station or inserted into cryoPREP for processing.
- Loosen the culture tube ¼ turn for venting to prevent rupturing the pouch during cryoPREP impact. Verify that the pouch is not swelled (a sign of trapped air) and that the sample remains centered in the pouch.
- Open the cryoPREP lid and quickly insert the previously frozen TT1 into the cryoPREP. 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 TT1.
- Close the cover, select the desired impact level (1 to 6), and press green “ACTIVATE” button. The cryoPREP hammer will impact and pulverize the sample. Higher number levels are meant for hard or large samples (e.g. bone). A typical setting for most samples is 3 or 4. The TT1 flexible pouch is designed to withstand *one impact*. If a second impact is needed to pulverize a sample, please see the note below and employ the TT1-XT tube.
- Raise the lid and grasp the culture tube to remove the TT1. Keep the TT1 with the pulverized sample on the bottom. To prevent melting and sample adhering to tube walls, immediately re-chill the TT1 and culture tube by placing them into the third position of the TT1 Prep Station.
- Once both tubes are chilled, the sample may be transferred to the glass culture tube. The sample may retain a flattened shape from the impact. Gently pinch the sides of the flexible pouch until the sample has broken into pieces small enough to pass through the TT1 opening. Quickly invert the tubes so the TT1 is on top and tap the pouch to transfer the tissue particles into the bottom of the culture tube. This step should be done quickly to avoid any melting and adhesion of sample to tube walls.
- Unscrew the TT1 from the tube and affix the culture tube cap to seal the culture tube. Discard the TT1 and its cap appropriately.

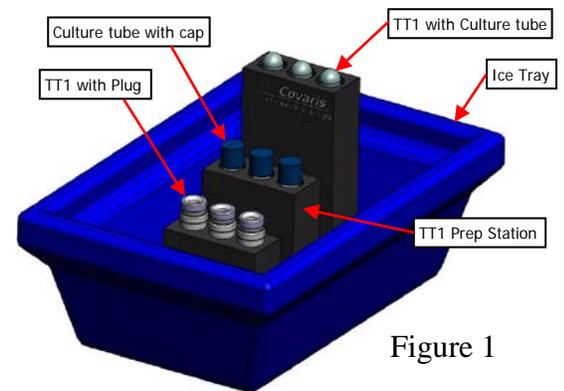
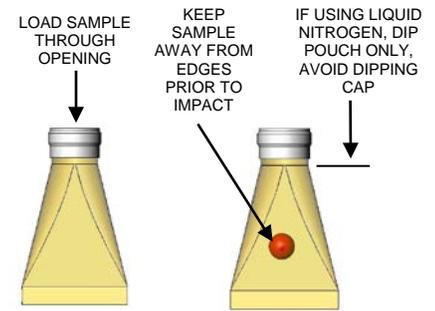


Figure 1



Application Notes

Impact Force – 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. Small mass tissue samples (e.g., biopsy) may be processed at impact level 1. Larger samples may require level 3 or 4.

Multiple Impacts - If a second impact is required to achieve complete pulverization, please employ the TT1-XT sample tube. After the first impact, carefully inspect the pouch for punctures. If a puncture is identified, transfer the sample to a new pouch before attempting a second impact. The TT1-XT designed to withstand up to two impacts under normal conditions. More than two impacts are not recommended.

Labels - Before processing samples, test labels for durability at cryogenic temperatures.

Sample Handling – Return pulverized samples to a cryogenic temperature until ready for AFA treatment to avoid sample degradation and adhesion to tube walls. RNA extraction requires immediate return of sample to a cryogenic environment.

Storage – After a sample has been pulverized, the TT1 may be used as a storage vessel by re-attaching plug TT1-P. Aliquots of the pulverized sample may be removed for analysis.