PRODUCT INSERT: tissueTUBEs for Small Samples (TT05, TT05M, TT05 XT and TT05M-XT)

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

TT05, TT05M, TT05XT and TT05M-XT tissueTUBEs 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

TT05, TT05M, TT05XT and TT05M-XT 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

Covaris warrants 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 TT05 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 TT05 tube is designed to allow easy transfer of the pulverized material to a 12mm diameter tissue vial. During subsequent treatment in Covaris AFA instruments homogenization times are reduced and the extraction efficiency of target bio-molecules improved, enabling larger sample size and higher throughput.

Equipment Notes

The TT05 tissueTUBEs 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 TT05 tissueTUBEs is held in the cryoPREP with a TT05 Insert (Covaris PN 500231) The CP02 must be equipped with a TT1 Holder (Covaris PN 500095) or TT2 Holder (Covaris PN 500096). The TT05 Insert fits into either of these two holders.

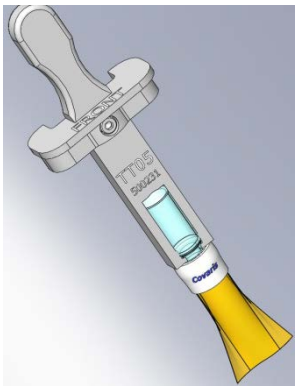
The TT05 / TT05M are designed for use with biological tissue samples up to 50 milligram masses. Appropriate tissues to use with the TT05 include liver, kidney, skeletal muscle, heart, dermal, lung, brain, and adipose.

The TT05XT/ TT05M-XT are a thicker walled tube designed to withstand greater stress. It 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 TT05 tube. Do not exceed 50 mg of mass.

The TT05 and TT05XT employ a 9-425 threaded top to mate with a 12x24mm glass tube (Covaris PN 520056). For applications that employ other tubes sizes, please contact Covaris.

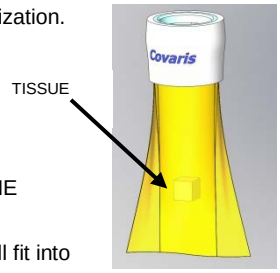
The TT05M and TT05M-XT employ a 10-425 threaded top to mate with milliTUBE 2 ml (Covaris PN 520132, 12x24mm glass tube) or milliTUBE 1 ml (Covaris PN520128,12x12mm glass tube). For applications that employ other tubes sizes, please contact Covaris.

Sample Processing Instructions

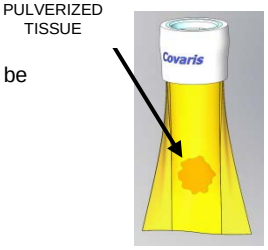


1. Place Ice tray with dry ice near the cryoPREP.
2. Previously labeled culture tubes can be pre-chilled by placing them into the dry ice.
3. Insert the sample specimen through the top opening of the TT05 as illustrated, using forceps or tweezers. Place the sample in the center of the flexible pouch, away from the edges.
4. After the sample is loaded, seal the TT05 by screwing a 12x24mm or 12x12mm tube into the top of the TT05. If multiple samples are being processed, they should be identified by the tube label or a tag on the TT05 cap. Do not label the flexible pouch.
5. Snap the culture tube into the TT05 Insert
6. While holding the TT05 Insert, freeze the sample by immersing the flexible pouch into a cryogenic environment (e.g., dry ice or liquid nitrogen). If using the TT05 Prep Station (PN500249) freeze the sample by inserting the flexible pouch into dry ice. 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.
7. When sample is frozen the sample can be inserted into cryoPREP for processing.
8. 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.

   THE TISSUE TUBE MUST BE VENTED DURING PROCESSING IN THE CRYOPREP BY LOOSENING THE CULTURE TUBE ¼ TURN.



9. Open the cryoPREP lid and quickly insert the previously frozen TT05 into the cryoPREP. The TT05 insert will fit into the TT1 or TT2 Holder, whichever is installed in the CP02 cryoPREP.
10. 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 TT05 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 TT05XT tube.
11. Raise the lid and grip the TT05 insert to remove the TT05. Keep the TT05 with the pulverized sample on the bottom. To prevent melting and sample adhering to tube walls, immediately re-chill the TT05 and culture tube by placing them into dry ice.
12. 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 TT05 opening. Quickly invert the tubes so the TT05 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.



13. Unscrew the TT05 from the tube and affix the culture tube cap to seal the culture tube. Discard the TT05 appropriately.

Application Notes

Impact Force – The mass of the sample and the degree of connective tissue will determine the impact force. For example, 10mg of liver will require low impact while 50mg 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 TT05XT/ TT05M-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 XT version 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 TT05 may be used as a storage vessel by attaching a culture tube. Aliquots of the pulverized sample may be removed for analysis.