

**PRODUCT INSERT: tissueTUBEs (TT2 and TT2-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**

TT2 tissueTUBEs and tissueTUBE Plugs (TT2-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**

TT2 and TT2-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](mailto:applicationsupport@covaris.com)

**INSTRUCTIONS FOR USE** *(Please refer to the cryoPREP User Manual for further instructions on use of this instrument)***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 TT2 tube and plug are designed to allow easy transfer of the pulverized material to a 20x125mm 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 TT2 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 TT2 Holder (PN/500096) to accept the TT2 tissue tubes.

The TT2 is designed for use with biological tissue samples < 2 gram mass. Appropriate tissue to use with the TT2 include: liver, kidney, skeletal muscle, heart, dermal, lung, brain, and adipose. Cell culture pellets are not appropriate.

The TT2 requires the use of a 20 x 125mm Culture Tube (a biologically inert threaded, hard borosilicate, round bottom glass tube with a writing patch and polypropylene cap). Covaris part number: 520012 or Fisher Scientific part number: 14-962-26K or VWR part number: 53283-685. For applications that employ other tubes sizes, please contact Covaris.

## Sample Processing Instructions:

- Place TT2 Prep station into ice tray and add dry ice.
- Previously labeled culture tubes can be pre-chilled by placing them into the second position in the TT2 Prep Station shown in Figure 1.
- Insert the sample specimen through the top opening of the TT2 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 TT2 by screwing a TT2-P plug or 20x125mm tube into the top of the TT2. If multiple samples are being processed, they should be identified by the tube label or a tag on the TT2 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 TT2 Prep Station; freeze the sample by inserting the flexible pouch into the first position in the TT2 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 the sample is frozen replace the plug with a pre-chilled culture tube. The sample can be placed in the third position in the TT2 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 TT2 into the sample holder until it reaches an internal “shelf.” This “shelf” ensures the sample is aligned in the impact zone of the TT2.
- 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 TT2 flexible pouch is designed to withstand up to *two impacts under normal conditions*. More than two impacts are not recommended.
- Raise the lid and grasp the culture tube to remove the TT2. Keep the TT2 with the pulverized sample on the bottom. To prevent melting and sample adhering to tube walls, immediately re-chill the TT2 and culture tube by placing them into the third position of the TT2 Prep Station. If a second impact is required to achieve complete pulverization, see note below and make sure the sample is still centered in the pouch and properly chilled. Repeat steps 8 and 9.
- 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 TT2 opening. Quickly invert the tubes so the TT2 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 TT2 from the tube and affix the culture tube cap to seal the culture tube. Discard the TT2 and its cap appropriately.

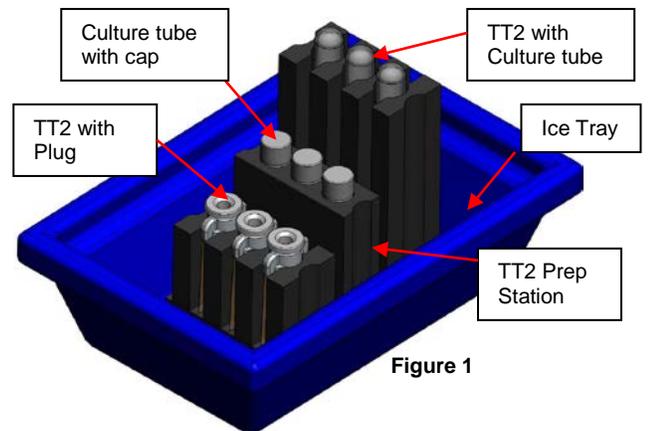
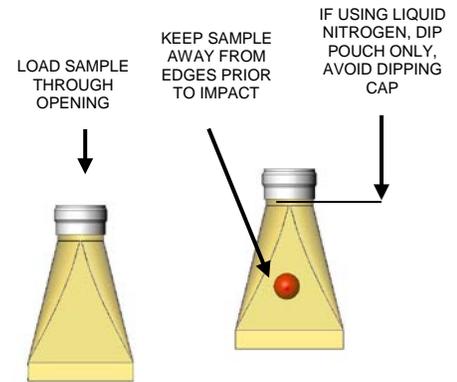
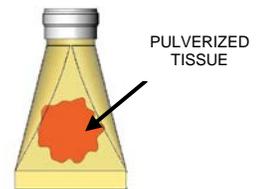


Figure 1



## 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 multiple impacts are required to achieve complete pulverization, try using a new TT2 and higher impact force. After each impact carefully inspect the pouch for punctures and that the sample is centered in the pouch. If a puncture is identified, transfer the sample to a new pouch before attempting a third impact.

**Labels** - Use the white writing patch or affix barcode and/or other labels to the culture tube to track the sample. The tissueTUBE pouch material is not designed for labeling. 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 TT2 may be used as a storage vessel by re-attaching plug TT2-P. Aliquots of the pulverized sample may be removed for analysis.