

TITLE: Tissue dry Pulverization using the CryoPrep™ CP02

Dry pulverization increases the tissue surface area and breaks up the extra-cellular matrix thus improving extraction efficiency of target biomolecules.

REQUIRED MATERIALS

1. Tissue samples
2. Liquid Nitrogen
3. Dry Ice
4. Tissue Tubes (please refer to the Table1 for selection and part #)
5. Transfer Tubes (please refer to the Table 2 for selection and part #)

STEP 1

Please use the Table 1 to choose the appropriate Tissue Tube based on the tissue mass.

TABLE 1

Sample mass	Tissue Tube	Plug	Tissue Tube Holder
50 mg or less	TT05 (PN 520071)		TT1 Holder (PN 500095)
50 mg or less hard tissue (heart, muscle, kidney)	TT05-XT (PN 520072)		TT1 Holder (PN 500095)
100 mg – 1 g	TT1 (PN 520001)	TT1-P (PN 520006)	TT1 Holder (PN 500095)
< 1g hard tissue (bone, seeds, tablets, plant material)	TT1-XT (PN 520007)	TT1-P (PN 520006)	TT1 Holder (PN 500095)
1-2 g	TT2 (PN 520021)	TT2-P (PN 520023)	TT2 Holder (PN 500096)

STEP 2

Please use the Table 2 to choose the appropriate Transfer Tube based on the extraction buffer volume.

TABLE 2

Buffer volume	Transfer Tube	Tissue Tube Adapter	S-series Processing Holder	E-series Processing Rack
300 µl-1.5 ml	TC 12x24 (PN 520056)	TT1ADP12* (PN 520062)	THQ12x24 (PN 500199)	TR12x24 (PN 500203)
1.0 ml-2.0 ml	TC 13 (PN 520010)	TT1ADP13 (PN 520017)	THQ13 (PN 500011)	TR2413 (PN 500033)
2.0 ml-3.0 ml	TC 16	N/A	THQ16	TR1216

Part number: 010303

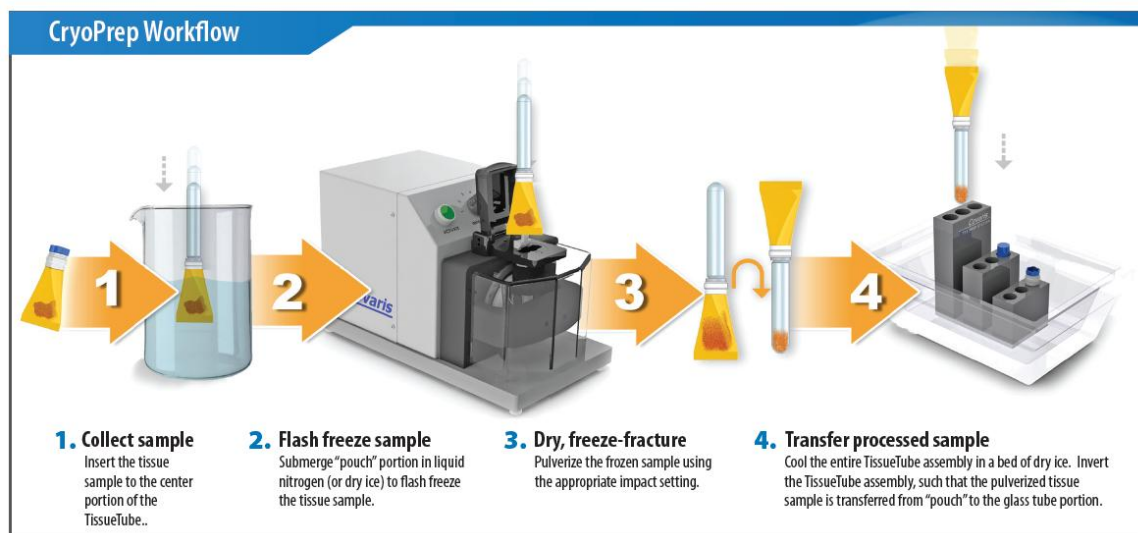
Revision: A

Date: 22 June 2015

	(PN 520011)		(PN 500012)	(PN 500031)
2.5 ml-5 ml	TC 20 (PN 520012)	N/A	THQ20 (PN 500051)	TR1220 (PN 500032)

* Adapter is only required for the TT1 and TT1-XT tissue tubes. Not required for TT05 and TT05-XT tissue tubes.

CryoPrep™ Workflow Chart



STEP 3 : Sample Processing

- 1. Cool the Prep Station** - Place TT1 or TT2 Prep Station into ice tray and add dry ice.
- 2. Pre-chill the Transfer Tube** – small transfer tubes (TC 12x24 and TC 13) can be placed directly on dry ice, whereas larger transfer tubes (TC 16 and TC 20) can be pre-chilled by placing them into the second position in the Prep Station shown in Figure 1.
- 3. Load Sample into the Tissue Tube** - Insert the sample through the top opening of the Tissue Tube as illustrated in Figure 2, using forceps or tweezers. Place the sample in the center of the flexible pouch, away from the edges.
- 4. Seal the Tissue Tube** - After the sample is loaded, seal the Tissue Tube by screwing either a plug or a transfer tube into the top of the Tissue Tube. If multiple samples are being processed, they should be identified by the tube label or a tag on the Tissue Tube Plug. Do not label the flexible pouch.
- 5. Snap Freeze the sample** - While holding either the plug or the transfer tube, freeze the sample by immersing the flexible pouch into a cryogenic environment (e.g., dry ice or liquid nitrogen). When using liquid nitrogen, dip only the sample pouch and avoid dipping the cap or transfer tube. The sample may now be stored at -80°C or in dry ice prior to pulverization.
- 6. Attach Transfer Tube** – If the Tissue Tube was sealed with a plug, replace the plug with a pre-chilled transfer tube.

Part number: 010303

Revision: A

Date: 22 June 2015

7. **Vent Transfer Tube** - Loosen the transfer 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.
8. **Load into CryoPrep** - Open the CryoPrep lid and quickly insert the previously frozen Tissue Tube 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 Tissue Tube. Use the special handle (PN 500231) for the TC12x 24 transfer tubes.
9. **Operate CryoPrep to deliver impact** - 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 Tissue Tube flexible pouch is designed to withstand *one impact*. If a second impact is needed to pulverize a sample, please see the note 2 below and employ the Tissue Tube-XT. Re-freeze the sample before delivering the second impact.
10. **Remove Tissue Tube with pulverized sample** -Raise the lid and grasp the transfer tube to remove the Tissue Tube. Keep the Tissue Tube with the pulverized sample on the bottom. To prevent melting and sample adhering to tube walls, immediately re-chill the Tissue Tube and transfer tube by placing them into the third position of the Prep Station or by placing them on a dry ice.
11. **Transfer pulverized sample to transfer tube**- Once both tubes are chilled, the sample may be transferred from the Tissue Tube to the transfer 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 Tissue Tube opening. Quickly invert the tubes so the Tissue Tube is on top and tap the pouch to transfer the tissue particles into the bottom of the transfer tube. This step should be done quickly to avoid any melting and adhesion of sample to tube walls.
12. **Store sample** - Unscrew the Tissue Tube from the Transfer tube and affix the cap to seal the transfer tube. Discard the Tissue Tube and its cap (if used) appropriately.
13. **Process sample**- Add the buffer to the transfer tube and place it in Covaris S or E series instrument.

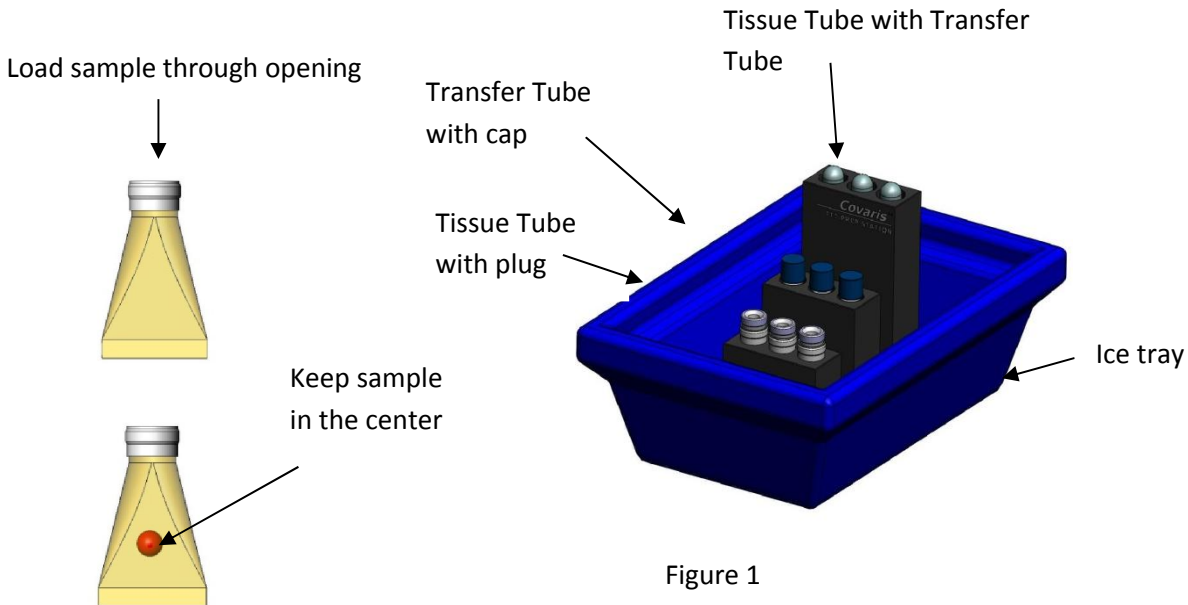


Figure 1
The TT1 Prep Station

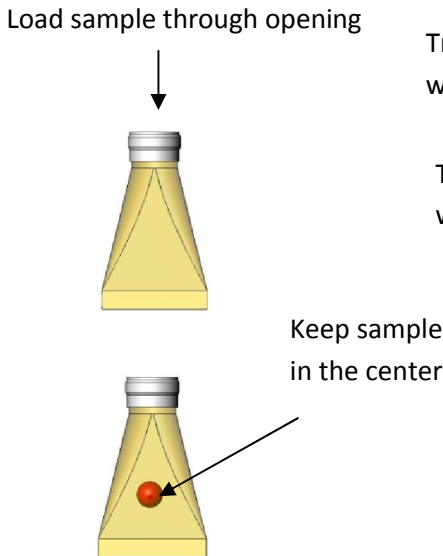


Figure 2
Loading sample into Tissue Tube



Figure 3
Pulverized Tissue

NOTES

Part number: 010303
 Revision: A
 Date: 22 June 2015

- 1.) **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.
- 2.) **Multiple Impacts** - If a second impact is required to achieve complete pulverization, please employ the TT1-XT or TT05-XT Tissue 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 and TT05-XT are designed to withstand up to two impacts under normal conditions. More than two impacts are not recommended. **Important** - After the first impact re-freeze the sample by dipping the flexible pouch in liquid nitrogen.
- 3.) **Labels** - Before processing samples, test labels for durability at cryogenic temperatures.
- 4.) **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 or addition of the extraction buffer followed by AFA treatment.
- 5.) **Storage** – After a sample has been pulverized, the Tissue Tubes may be used as a storage vessel by re-attaching plug. Aliquots of the pulverized sample may be removed for analysis.