CRYOXTRACT™
An Allied Minds Company

CXT 350
FROZEN SAMPLE ALIQUOTTER

Benefits:

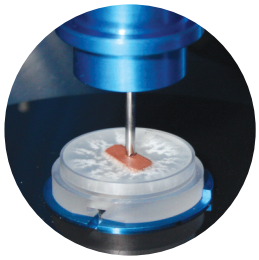
- Provides better and repeated access for all types of frozen tissue and biofluid samples
- Optimizes data by protecting samples from temperature cycling (freeze/thaws)
- Targets and aliquots specific regions of frozen tissue specimens easily
- Streamlines laboratory workflows and increases aliquotting efficiencies
- Eliminates the safety problems associated with manual access methods

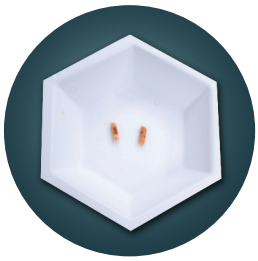
Features:

- Proprietary coring system
- Laser targeting system for accurate coring
- LN_2-based chilling system
- Proprietary single-use coring probe and chilling fixtures
- Chilled sample and destination tube fixtures

Optimize Biospecimen Integrity

Provide better sample access and maximize scientific outcomes





The **CXT 350** Frozen Sample Aliquotter supports a widespread need for targeted, repeated and safe access to frozen samples to advance research and discovery. The system allows for uniform and efficient distribution of frozen tissue and biofluid aliquots, maximizing sample integrity and optimizing scientific outcomes. The CXT 350 provides unprecedented access to samples while maintaining their frozen state. This ensures the quality of both the primary and aliquotted sample, eliminating their potential degradation due to thawing.

The compact benchtop design, and LN_2 chilling, allows for integration into standard laboratory workflows. For tissue samples, the unique mounting method enables standard H&E slides and a pathology review to target specific regions of interest within the sample.

The frozen coring process includes pre-chilled coring probes and destination tubes designed to ensure the frozen state is maintained throughout the process. Proprietary single-use, nuclease-free, disposable coring probes eliminate the potential for sample-to-sample carryover.

CryoXtract Instruments | 5 Constitution Way | Woburn MA 01801 USA
+01 339.298.5615 | info@cryoxtract.com | **www.cryoxtract.com**

RNA Extraction from Frozen Tissue

The Eastern Division of the Cooperative Human Tissue Network (CHTN) completed a proof-of-concept study testing the feasibility for the utilization of Cryoxtract's proprietary frozen aliquotting technology with frozen tissue samples. This study utilized human uterine tissue surgically procured from 4 donors. Tissue samples were snap frozen as part of the procurement process in close proximity to the surgical procedure. Samples were stored at -80°C prior to and throughout the execution of the study.

As part of the study, two post-procurement sampling techniques were compared over a course of 4 sampling rounds (once per day). Uterine tissue from 4 donors was sampled in a frozen state (no thawing) manually by scalpel (n = 16 slices) and by frozen aliquotting (n = 16 cores). Frozen slices and cores were processed for total RNA using standard RNA tissue extraction protocols and commercially available kits. RNA integrity (RQI) was then assessed on the Bio-Rad Experion system.

RNA Quality

Figure 1 compares the average RQI score (donors 1-4) for each sampling technique over 4 sampling rounds. The study demonstrated that high quality total RNA was recovered from cores of frozen human uterine tissue produced via frozen aliquotting. More so, RNA integrity was maintained in frozen human uterine samples when accessed over multiple discrete rounds of frozen aliquotting. The study also shows that when benchmarked against established post-procurement sampling techniques (frozen slicing), frozen aliquotting resulted in comparable RNA integrity for extracted total RNA.

Figure 1: Frozen Aliquotting vs. Frozen Slicing of Human Uterine Tissue

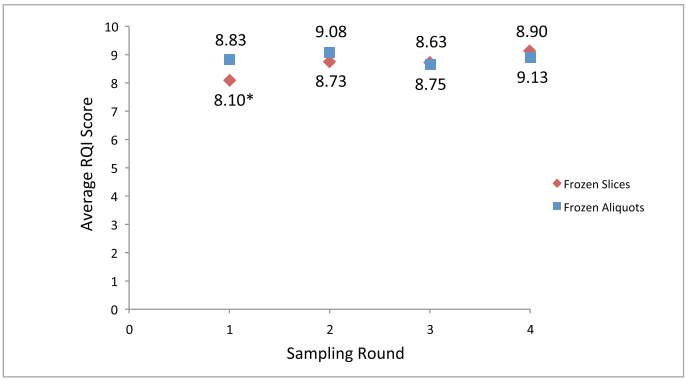


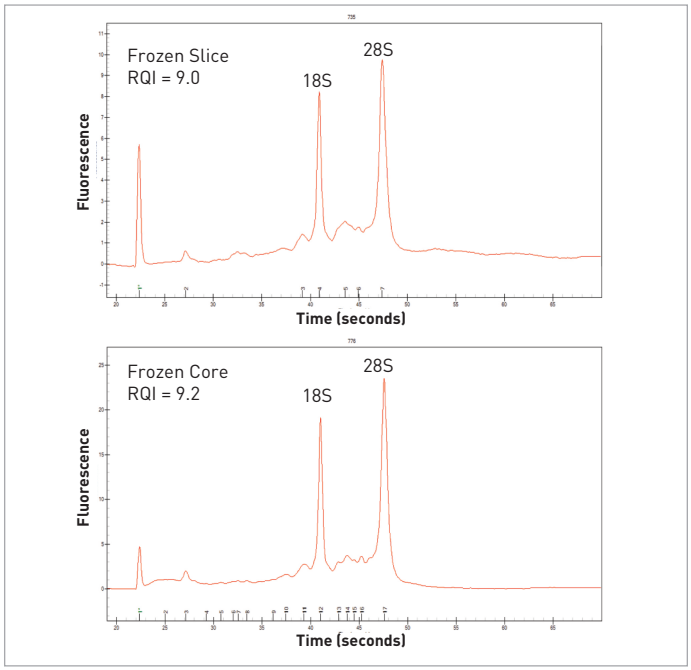
Table 1: RQI Score Summary Table

	Donor 1		Donor 2		Donor 3		Donor 4	
	Slices	Cores	Slices	Cores	Slices	Cores	Slices	Cores
Round 1	7.6	8.6	7.9	8.8	9.1	8.4	7.8	9.5
Round 2	7.5	8.3	8.6	9.4	9.1	9.2	9.7	9.4
Round 3	8.6	8.6	9.0	7.7	8.1	9.0	9.3	9.2
Round 4	2.7*	8.4	9.5	9.1	8.4	8.6	9.5	9.5

* Outlier removed from donor 1 average RQI

Figure 2 shows an electropherogram representative of the total median RQI score for each sampling technique. The clear and strong presence of ribosomal RNA subunits (18s/28s) is regarded as strong indicator of RNA integrity. Generally, all electropherograms produced in this study showed comparable profiles to those in Figure 2.

Figure 2: Representative Electropherograms for Total RNA



RNA Yield

Table 1 summarizes RNA yields based upon the total amount of RNA recovered (nanograms) vs. the amount of uterine tissue (milligrams) utilized for extraction. RNA yields are reported as the average yield produced per donor (rounds 1-3) for each sampling technique. The total average RNA yield for each sampling technique is also displayed. Overall, RNA yields for both sampling techniques were deemed acceptable.

Table 2: Total RNA Yield Summary Table (nanograms RNA/ milligrams tissue)

	Donor 1	Donor 2	Donor 3	Donor 4	Total
Slices	157	116	102	94	117
Cores	164	230	99	255	187

Study Conclusions

When compared to an accepted and widely utilized sampling technique for frozen tissue, frozen aliquotting was observed to perform equally as well and resulted in high quality total RNA with acceptable yields.