High-Throughput Proteomics Enabled by Covaris AFA® Technology: A Scalable & Streamlined Sample Preparation Workflow for Fresh Frozen Tissue Analysis

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Overview

We present a high-throughput workflow for Fresh Frozen (FF) tissue sample preparation using Covaris Adaptive Focused Acoustics® (AFA®) technology, enabling robust, fast, and scalable protein extraction with a 1 hour accelerated trypsin digestion.

Introduction

Comprehensive Sample Preparation Workflow for Proteomic Analysis with Fresh Frozen Tissues

- Low-input fresh frozen tissue proteomics accelerates drug development by enabling protein analysis in small samples, providing valuable data for pharmacokinetics – pharmacodynamics analysis, biomarker discovery, and mechanistic disease insights.
- Protein extraction faces challenges like variability, degradation, and non-standardized protocols. MS-based proteomic assays in drug studies can greatly benefit from efficient, scalable, & high-throughput workflows.
- The Covaris R230's AFA technology employs focused-ultrasonication for efficient tissue processing, enabling complete homogenization and digestion within 8 hours for same-day analysis using a 96-well format.

Method

Figure 1. Covaris Adaptive Focused Acoustics Technology. The Covaris high-throughput R230 Focused-ultrasonicator employs AFA for diverse protein applications.

- **Sample Prep:** The Covaris R230 Focused-ultrasonicator was used to process 1–10 mg of fresh frozen (FF) tissue in microTUBE-130 with Bead Snap-Cap (Covaris PN 520360) and Tissue Lysis Buffer (Covaris PN 520284). Liver, small intestine, and lung tissue samples underwent AFA treatment, followed by centrifugation and supernatant collection. Eight (8) samples were homogenized in parallel at 10°C in continuous mode for 5–15 minutes, depending on tissue input. Protein concentration was determined via BCA assay. Downstream cleanup using Protein Aggregation Capture (PAC), and AFA-enabled expedited digestion for peptide generation was performed in a 96 AFA-TUBE TPX Plate (Covaris PN 520291).

- **LC-MS:** Samples were run using EvoSep One/Bruker timsTOF HT system. Samples were desalted using EvoTip Pure C18 desalting columns. They were run on a 30 SPD method (44-minute gradient) with separation occurring on a PepSep Series C18 column 15 cm x 150 µm, 1.5 µm. Of each sample, 500 ng was injected on column. Data were filtered for a FDR of 1 % using Bruker Proteoscape software.

Workflow and Results

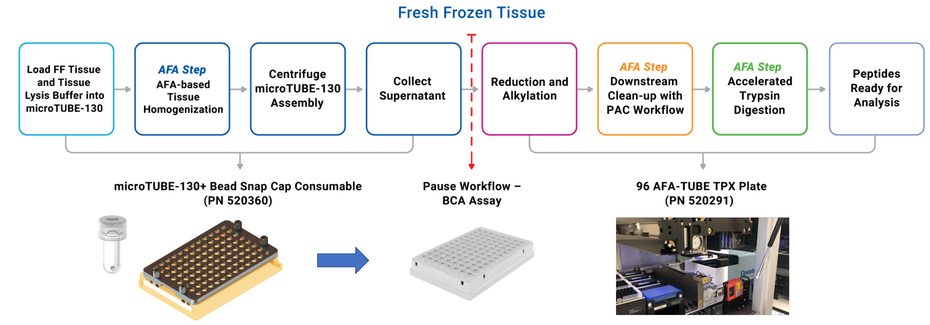

Figure 2. Workflow and Consumable Format. Tissue Homogenization is carried out in microTUBE-130 Bead Snap-Cap tubes. Reduction, alkylation, magnetic based purification, as well as AFA-enabled accelerated trypsin digestion is carried out in 96 AFA-TUBE TPX Plate for semi-automation.

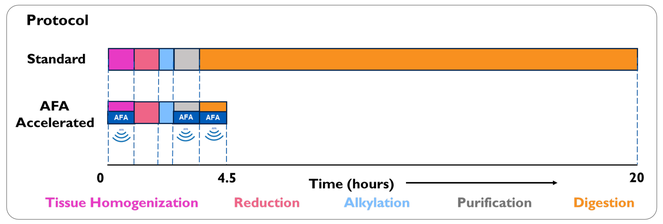

Figure 3. AFA-enabled Workflow Efficiency. The optimized protocol reduces tissue processing—from homogenization through peptide digestion—to under 5 hours with 5mg tissue input while maintaining high throughput.

Figure 5. AFA-enabled efficient protein extraction. Average normalized protein yield ranged between ~60–160 mg protein /mg tissue. Tests were performed with different tissue types (Liver, Lung, Small Intestine), and different tissue input amounts (2, 5, and 10mg) each with 4 technical replicates.

Sample Type	Input Amount (mg)	Average ug/mg tissue	%CV
Liver	2	155.5	15.1
	5	116	15.4
	10	102	13.0
Small Intestine	2	99	10.5
	5	80	16.4
	10	68.8	6.7
Lung	2	91.8	19.2
	5	85.8	6.1
	10	61.8	13.6

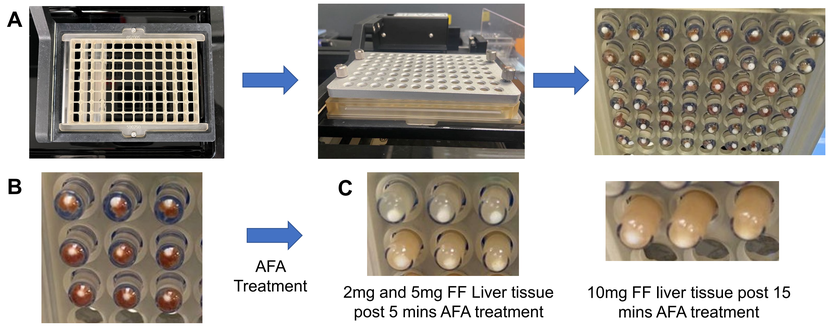

Figure 4. R230 Setup with microTUBE-130 Bead Snap-cap for FF Tissue Homogenization. **A**) R230 rack, 96-well plate format viewed from the top and the bottom. **B**) Close up image of 2, 5, and 10mg FF tissue before AFA processing. **C**) 2 & 5 mg tissue homogenized after 5 mins AFA treatment, 10 mg tissue homogenized after 15 mins. AFA treatment. Tissue homogenization is consistent across a wide input range of tissue samples.

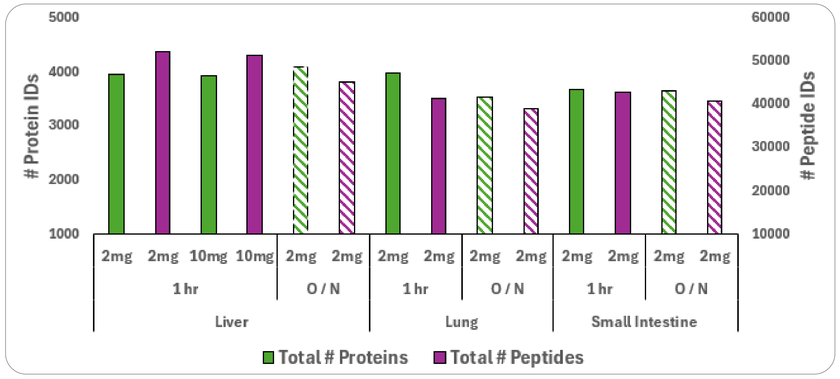

Figure 6. Equivalent Performance Between AFA Accelerated 1-hr Digestion and Standard Overnight Digestion. Protein and peptide IDs are similar across tissue types, tissue input amounts, and with both AFA-enabled accelerated 1-hour digestion and standard overnight digestion.

Discussion

- The consistently high protein yields—exceeding 50 µg protein/mg tissue across various tissue types and input quantities—demonstrate the versatility and robustness of this extraction method.
- Our protocol, combining AFA-assisted homogenization with a shortened one-hour AFA-enabled accelerated trypsin digestion, achieved comparable results to traditional processing methods.
- The enhanced processing speed and higher sample throughput make this low input FF tissue proteomics workflow suitable for drug development applications.
- R230 compatibility with different liquid handlers enables wide- spread semi-automation integration in different labs.

Conclusions

- The high-throughput Covaris AFA-enabled workflow ensures a robust, reproducible, and fast protein analysis assay for fresh frozen tissues without compromising analytical quality and reliability.
- This streamlined protocol will enhance productivity for any laboratory focusing on protein analysis starting with any complex biological matrix. This workflow delivers consistent, high-quality results regardless of sample type or application.

Acknowledgements

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