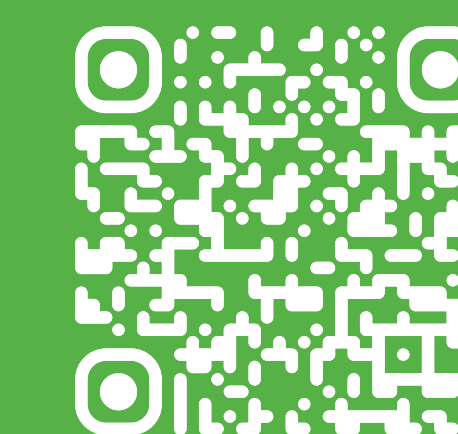




Overview

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 FF tissue proteomics supports drug development by enabling protein analysis for PK/PD, biomarkers, and disease insights.
- Protein extraction is challenged by variability, degradation, and lack of standardization; MS-based assays benefit from scalable, high-throughput workflows.
- Covaris R230 with AFA Focused-ultrasonication enables full tissue homogenization and digestion within 8 hours for same-day 96-well analysis.

Methods

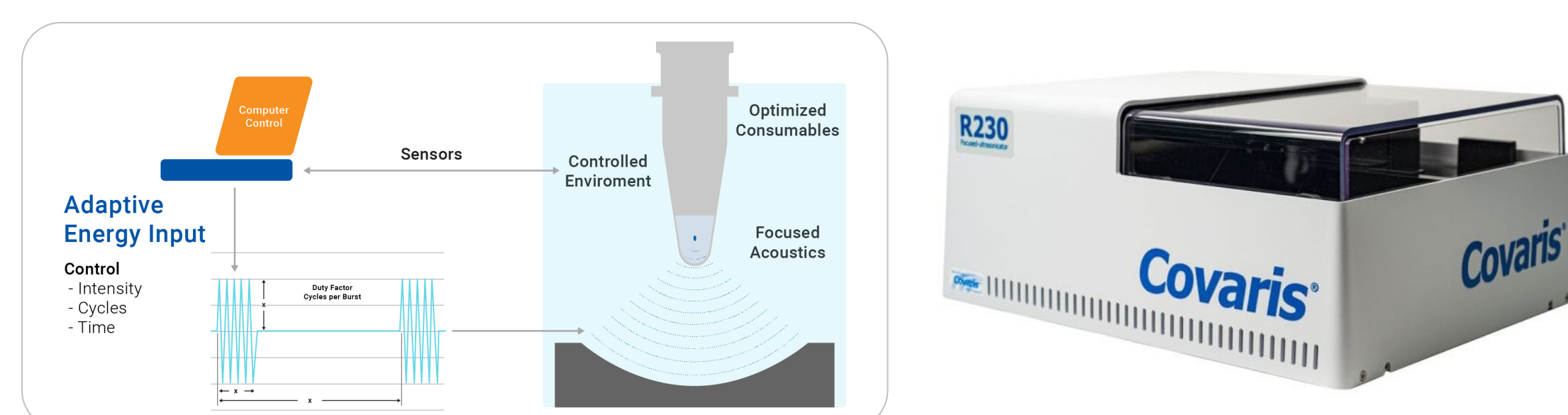


Figure 1. Covaris Adaptive Focused Acoustics Technology. The Covaris R230 high-throughput Focused-ultrasonicator employs AFA for diverse protein applications.

Sample Prep: Covaris R230 Focused-ultrasonicator was used to process 1–10 mg FF tissue in microTUBE-130 (PN 520360) with Tissue Lysis Buffer (PN 520284). Liver, small intestine, and lung samples underwent AFA treatment, 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 Proteome software.

Workflow and Results

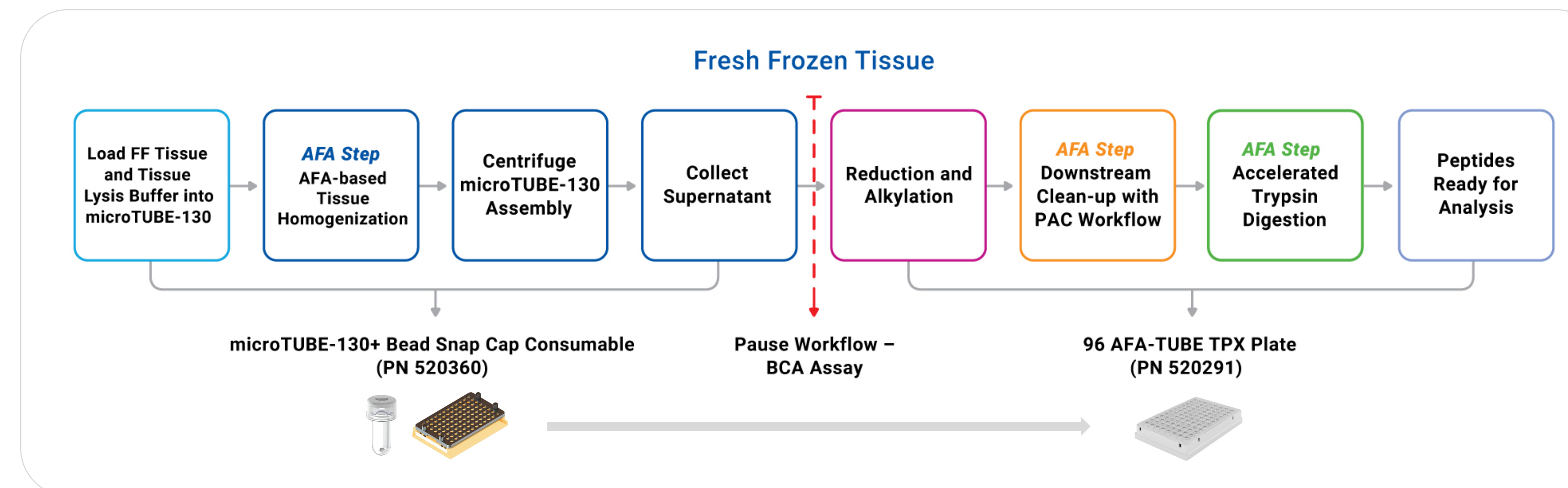


Figure 2. Workflow and Consumable Format. Tissue Homogenization is carried out in microTUBE-130 Bead Snap-Cap tubes. Reduction, alkylation, magnetic based purification, as well as AFA-enabled accelerated trypsin digestion is carried out in 96 AFA-TUBE TPX Plate.

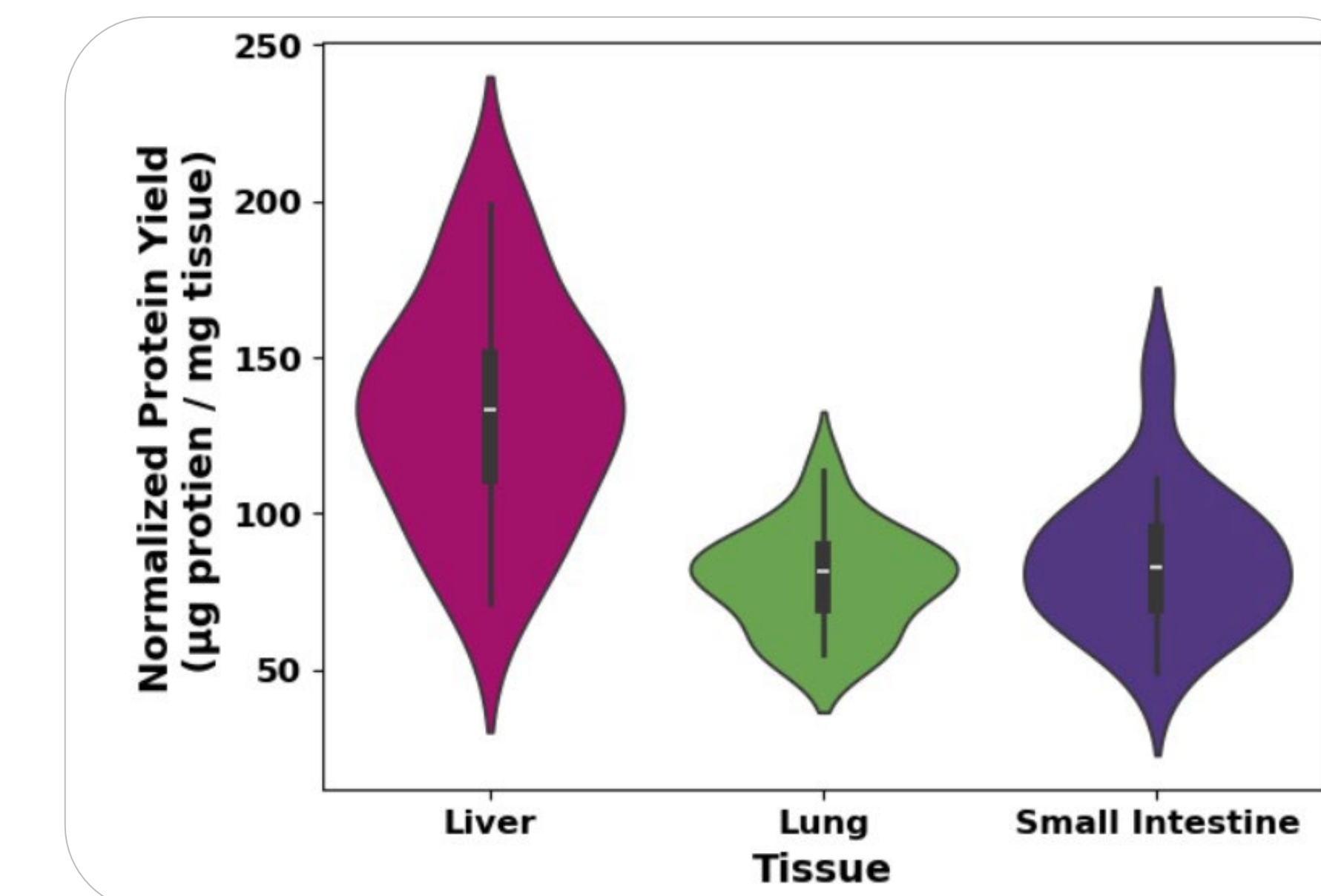


Figure 4. AFA-enabled Efficient Protein Extraction. Average normalized protein yield ranged between ~ 60 – 160 µg protein/mg tissue. Tests were performed with different tissue types (Liver, Lung, Small Intestine), and different tissue input amounts (2, 5, & 10 mg) each with 4 technical replicates.

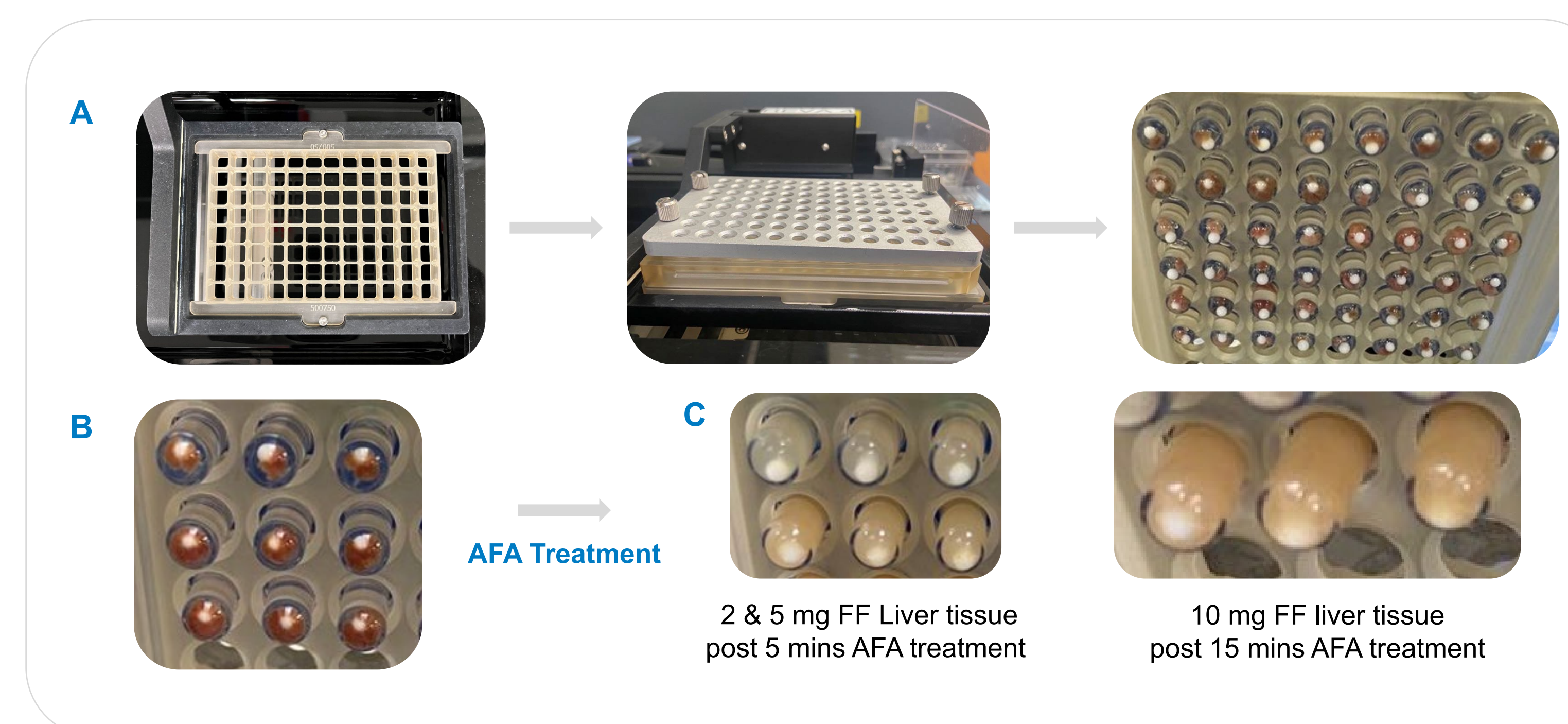


Figure 3. R230 Setup with microTUBE-130 Bead Snap-cap for FF Tissue Homogenization. A) R230 rack, 96-well plate format viewed from the top and the bottom. B) Close up image of 2, 5, & 10 mg FF tissue before AFA processing. C) 2 & 5 mg tissue homogenized after 5 mins AFA treatment, 10 mg tissue homogenized after 15 mins AFA treatment. Tissue homogenization is consistent across a wide input range of tissue samples.

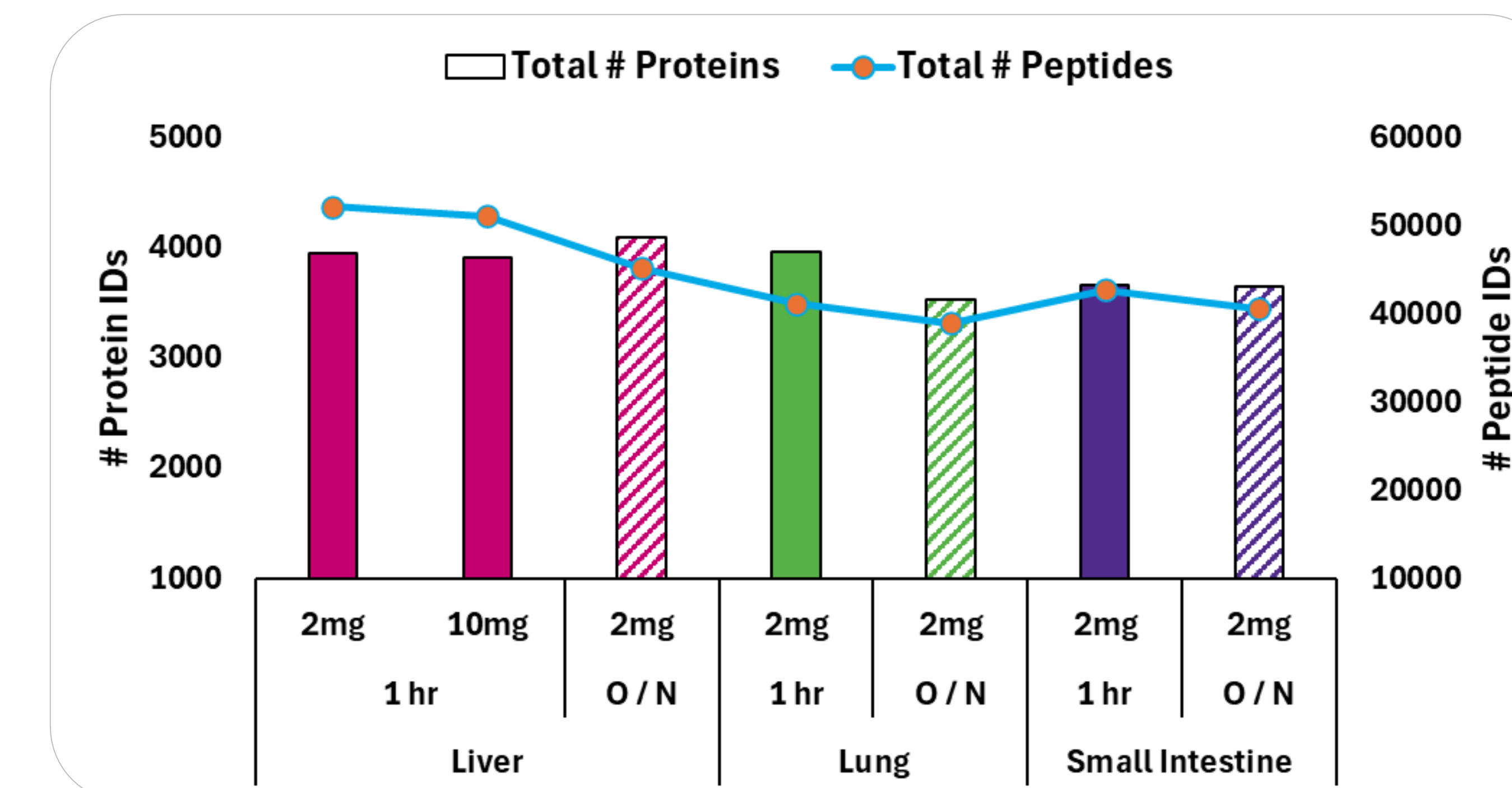


Figure 5. Equivalent Performance Between AFA-accelerated, 1-hour, and Standard Overnight Digestion. Protein and peptide IDs are similar across tissue types, tissue input amounts, and with both AFA-enabled accelerated 1-hour digestion and standard overnight digestion.

Workflow Efficiency

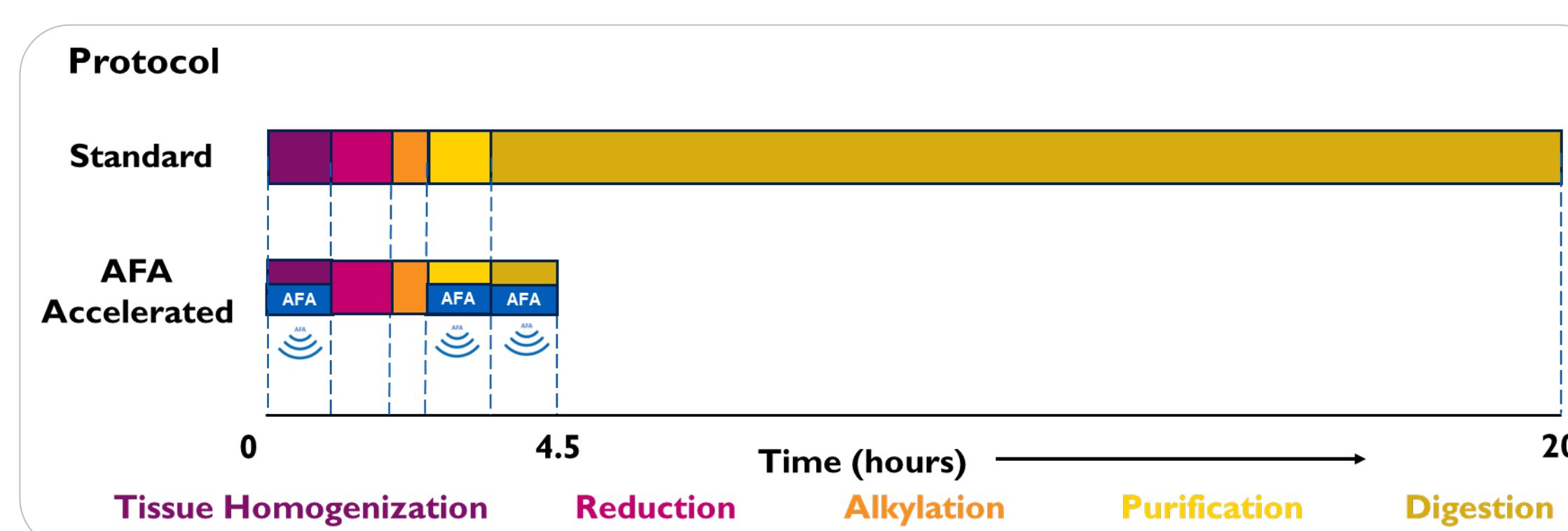


Figure 6. AFA-enabled Workflow Efficiency. The optimized protocol reduces tissue processing, from homogenization through peptide digestion, to under 5 hours with 5 mg tissue input while maintaining high throughput.

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 1-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.

Conclusion

- 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 different tissue types, input amounts, or varying throughput requirements.