# Better Sample Preparation for Confident Data - Extraction, Purification, & Accelerated Digestion of Proteins from Mammalian Cells

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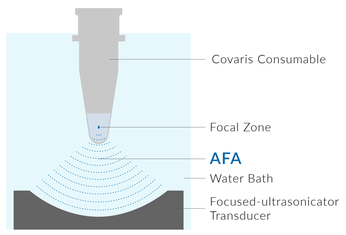
## Introduction and Background

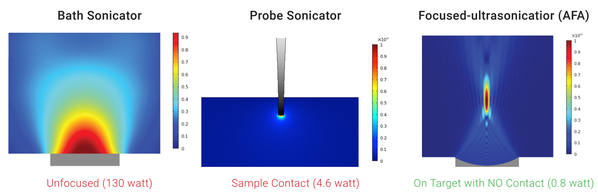
Over the last twenty years, breakthroughs in analytical technologies like LC-MS have transformed proteomics from a purely research-focused area to a field with significant applications in clinical research and biopharma, directly benefiting patients. However, achieving high-quality proteomics data is not possible without a robust sample preparation protocol. Adaptive Focused Acoustics® (AFA®) Technology has been extensively used in genomics and recently has found widespread use in efficient extraction, purification, and expedited digestion of proteins from complex matrices.

In this study, we report development of a comprehensive workflow with the Covaris truPREP® Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion) that ensures extraction, purification, and accelerated digestion for a wide range of mammalian cells.




**Covaris Technology:**
- Less Energy
- More Control
- Maintain Isothermal Process

**Characteristics of Adaptive Focused Acoustics (AFA) Technology.** High-frequency acoustic energy is precisely focused onto the sample, minimizing energy waste that would otherwise cause sample heating, and thereby maximizing its impact on the sample. Thanks to sophisticated technology, energy can be controlled, enabling the user to tune AFA conditions for their specific needs, e.g., cell disruption (high energy) or gentle mixing (low energy).




## Workflow

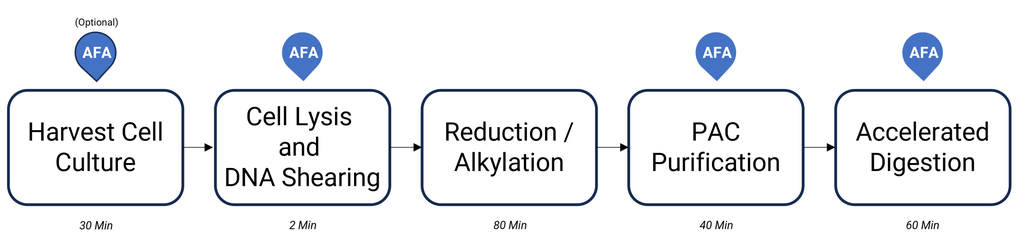



**Figure 2. Schematic truPREP Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion) workflow diagram.** This streamlined sample preparation workflow can be performed in a single shift, starting with cell culture and ending with peptides in just 4 hours. Cell lysis and DNA shearing are performed at the same time in batches of 8 or 16 samples with the 8 AFA-TUBE TPX Strip in the R230 Focused-ultrasonicator. The resulting lysate is reduced/alkylated prior to Protein Aggregation Capture (PAC), which is enhanced with AFA. Following purification, accelerated trypsin digestion is performed in ammonium bicarbonate buffer in under 1 hour with AFA treatment.

## Methods

Adherent cells were detached from 96-well polystyrene cell culture plates either with AFA and 1X PBS (25 µL) or standard trypsinization. Cells were transferred into 8 AFA-TUBE TPX Strips, and 25 µL lysis buffer was added followed by cell lysis and DNA shearing with AFA in a Covaris R230 Focused-ultrasonicator. Samples were reduced and alkylated prior to protein purification, which was conducted adapting the PAC protocol (Hughes, C.S., Moggridge, S., Müller, T. et al. Single-pot, solid-phase-enhanced sample preparation for proteomics experiments. Nat Protoc 14, 68–85 (2019)). The beads were then suspended in Trypsin buffer for either accelerated on-bead digestion under 1 hour with AFA treatment or at different time points including overnight digestion in a heat block.

Extraction was performed with a range of different cell counts, with 2 adherent and 1 suspension cell line, and with a series of trypsin ratios. Stability and reproducibility of the truPREP Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion) was also evaluated over the course of several months.

The samples were first desalted on a Thermo Fisher Scientific Acclaim™ PepMap™ 100 C18 HPLC column (3 µm particle size, 75 µm x 2 cm, 100 Å) prior to separation on a Thermo Fisher Scientific PepMap RSLC C18 EASY-Spray™ Column (3 µm particle size, 75 µm x 15 cm, 100 Å). An Orbitrap Fusion™ Lumos™ mass spectrometer (Thermo Fisher, San Jose, CA, USA) coupled online to an EASY-nLC 1200 (Thermo Fisher, San Jose, CA, USA) was used to perform the RP-nLC/ESI-MS analysis.

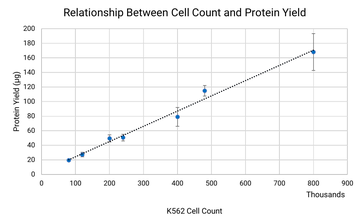



**Figure 3. Protein Yield vs. Cell Count.** Cultured K562 was serially diluted from 800,000 down to 80,000 cells prior to sample preparation with the truPREP Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion). A strong correlation was observed between cell count & protein yield, with an R2 of 0.987, which demonstrates the range & reliability of Covaris' lysis and extraction workflow.

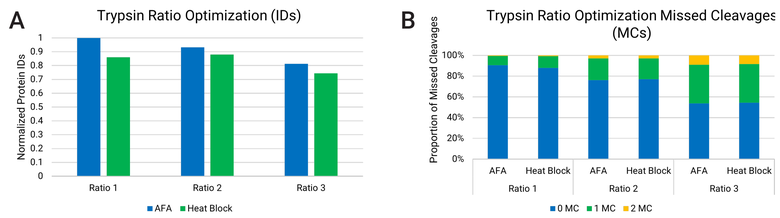



**Figure 4. Trypsin Ratio Optimization.** A commercially prepared intact human (K562) protein extract (Promega® V6941) was digested with different ratios of Trypsin Gold (Promega V52870) for 1 hour using either a heat block or AFA for temperature control. Samples were analyzed using LC with high resolution accurate MS. While missed cleavages increased at lower enzyme ratios, higher protein coverage was observed for AFA-mediated digestion as compared to heat block.

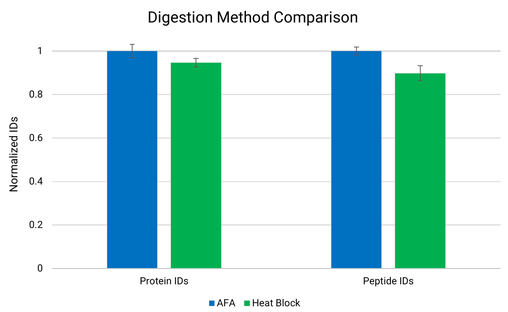



**Figure 5. Digestion Method Comparison.** A commercially prepared intact human (K562) protein extract (Promega V6941) was digested on-bead with Trypsin Gold (Promega V52870) for 1 hour using AFA, and for 18 hours using a heat block. Samples were analyzed using LC with high resolution accurate MS. Expedited digestion with AFA resulted in significantly higher digestion efficiency compared to overnight digestion without AFA. A higher number of protein and peptide identifications was obtained from the AFA-assisted method with higher reproducibility and lower coefficient of variation.

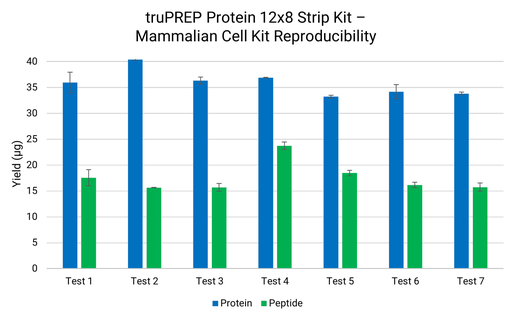



**Figure 6. High Reproducibility with truPREP.** The truPREP Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion) was utilized to isolate protein and prepare peptide digests from 100,000 K562 cells several weeks to several months apart for the purpose of testing stability and reproducibility of results. Total protein yields were measured to be above 33 µg for all trials, with CVs below 6%. Total peptide yields were consistently observed to exceed 15 µg for all trials, with CVs below 10%.

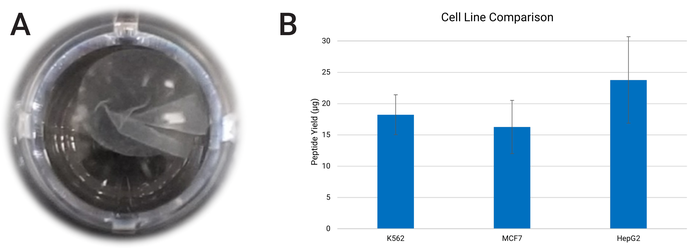



**Figure 7. Compatibility with Adherent and Suspension Cells.** A comparison between peptide yields from adherent and suspension cell lines. Fully confluent MCF7 and HepG2 cells were harvested in their entirety by using AFA for detachment of the monolayer (**A**). K562 cells were quantified with the Luna-FL™ fluorescence cell counter to enable transfer of 100,000 cells per replicate. Proteins were extracted successfully, resulting in peptide yields above 15 µg for all cell lines (**B**).

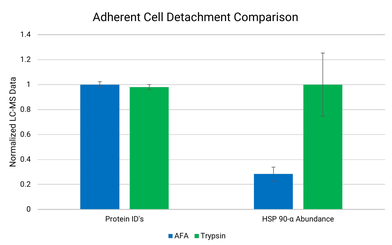



**Figure 8. Adherent Cell Detachment.** Adherent human colorectal carcinoma cells (HCT 116) were grown to 60-70% confluency and harvested either with standard trypsinization or with AFA-mediated detachment. Samples were analyzed using LC with high resolution accurate MS. While total protein identifications were similar, abundances of various proteins differed significantly. Extracts from trypsinized cells contained ~3.5x more HSP 90-α than AFA-treated cells, indicating a difference in stress response as a result of enzymatic detachment.

## Discussion and Conclusions

- Covaris AFA enables an automatable workflow starting from cell culture (adherent or suspension) to LC-MS ready protein hydrolysates.
- AFA simplifies adherent cell detachment by eliminating the need for cumbersome enzymatic incubation steps and improving user convenience.
- Following displacement of adherent cells, the entire workflow takes place in a Covaris 8 AFA-TUBE TPX Strip, limiting transfer steps to a total of 3 and enabling the AFA treatments necessary for subsequent steps.
- Cell lysis and DNA shearing result from a single AFA treatment, extracting proteins and eliminating viscosity due to high molecular weight nucleic acids.
- The PAC workflow facilitates sample preparation by enabling compatibility with different buffer systems and simplifying cleanup.



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