Streamlined FFPE Proteomics with Covaris AFA® and ProtiFi™ S-Trap™ Technologies

FFPE blocks represent one of the largest sources of archived clinical samples. Traditionally, due to inefficient or incomplete deparaffinization and decrosslinking, FFPE analysis has suffered from poor protein recovery, lack of reproducibility, and lack of speed. The unique combination of Covaris AFA and ProtiFi™ S-Traps™ allows for a rapid, streamlined approach using one tube and one column. This novel workflow affords the highest yields of protein and number of identifications and the most reproducible FFPE sample processing. In addition, it is well suited to high-throughput workflows.

Current Challenges

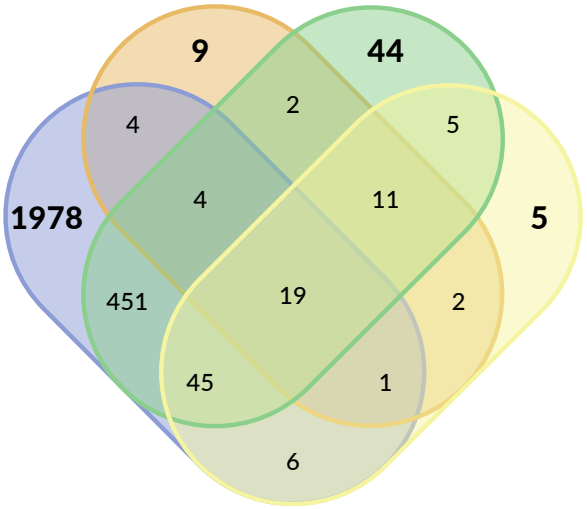
1. Current FFPE sample extraction and purification workflows are neither standardized nor high-throughput
2. Deparaffinization and sample hydration is slow and uses toxic organic solvents
3. Protein release and identification can be inefficient and irreproducible due to extensive molecular crosslinking from formalin fixation

The AFA/S-Trap™ Solution

1. Rapidly process up to 96 FFPE samples to peptides in less than 5 hours
2. Actively remove paraffin without organic solvents using AFA and SDS, then process samples to peptides with simple, easy-to-use ProtiFi™ S-Traps™
3. Consistently obtain the highest yield of the whole proteome with a robust two part workflow

The use of 5% sodium dodecyl sulfate (SDS), Covaris Adaptive Focused Acoustics® (AFA®) and ProtiFI™ S-Trap™ sample processing technology enables the highest protein yield and largest number of protein identifications. Aqueous buffers such as ammonium bicarbonate (NH_4HCO_3) were not effective to solubilize proteins. Bead beating (BB) was inferior to AFA as an extraction technique.

Rapidly Standardized Protein Extractions



	Extraction Buffer	Extraction Technique	Digestion Technique	Total Proteins ID'ed	% of proteins ID'ed with any condition
Blue	SDS	AFA	S-Trap™	2508	97.0%
Orange	NH_4HCO_3	AFA	S-Trap™	52	2.0%
Green	SDS	BB	S-Trap™	581	22.5%
Yellow	NH_4HCO_3	BB	S-Trap™	94	3.6%

Figure 1. Number of proteins identified using that particular extraction condition. FFPE block of human kidney; 10 µm scrolls. A fixed 0.5% of the total sample extracted by these conditions was analyzed by LCMS. AFA combined with S-Traps™ and 5% SDS afforded the highest recovery and identified 97% of the proteins detected by any condition. 100% represents the 2586 proteins identified with any extraction technique, of which 76% were identified solely with the SDS/AFA/S-Trap protocol.

Covaris®

PROTIFI™

Workflow

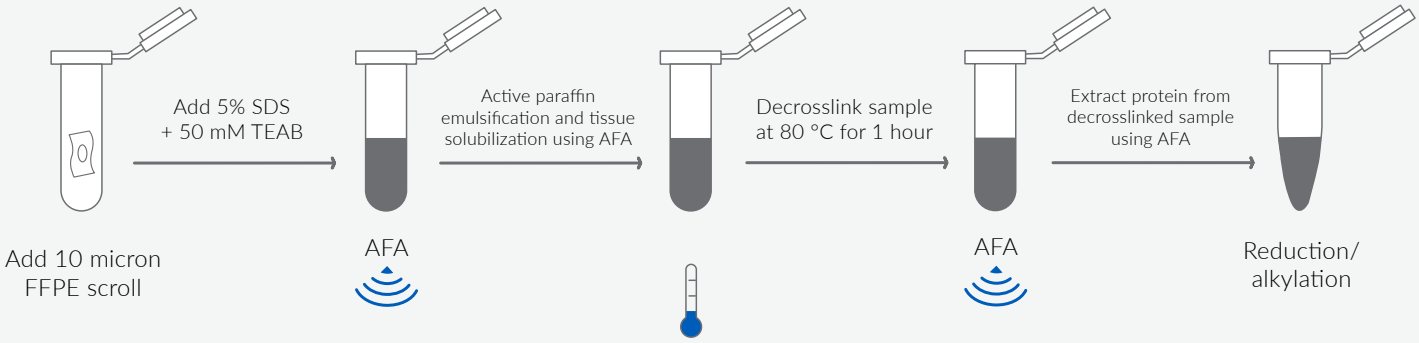


Fig 2. Steps of AFA sample processing

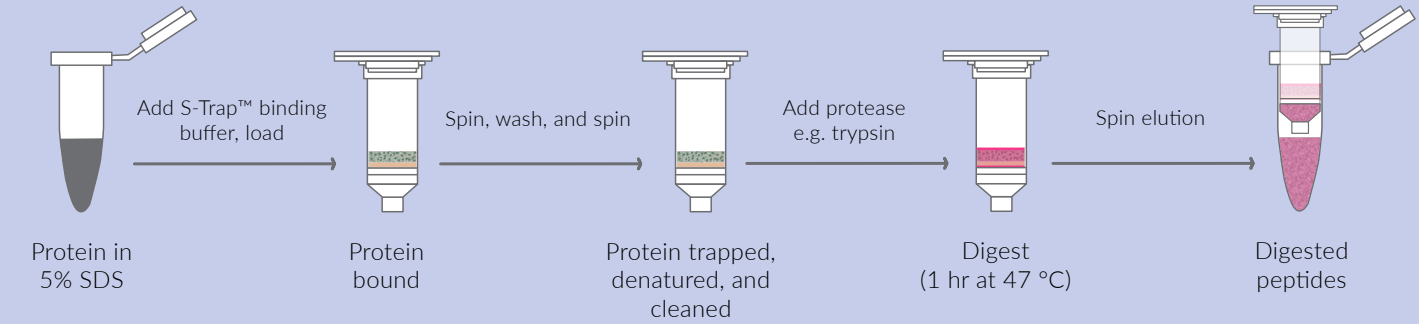


Fig 2. (cont) Steps of S-Trap™ sample processing

Suggested Products

Covaris

- Focused-ultrasonicator — M-Series, S-Series, E-Series, or LE-Series[1]
- microTUBE-130 with Fiber — PN 520216

ProtiFI™

- S-Trap™ micro (≤ 100 µg) — PN C02-micro-10, C02-micro-40, C02-micro-80, & K02-micro-10[2]
- S-Trap™ mini (100 to 300 µg) — PN C02-mini-10, C02-mini-40, & C02-mini-80, & K02-mini-10[2]

References

1. https://www.covaris.com/products-services/instruments
2. www.protifi.com/s-trap

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