EVER SIMPLER:
Streamlining Proteomics with S-Trap™ Turbo & BCA-No-More™

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INTRODUCTION

Recent advances in proteomics enable high-throughput sample identification and quantification, necessitating improved sample preparation workflows. To enhance throughput and robustness, simplifying workflows is crucial. The widely used S-Trap™ system has standardized proteomics sample preparation. Building on this, we introduce a streamlined workflow with S-Trap™ Turbo and BCA-No-More™ technologies to eliminate steps of protein quantification and concentration ***(Fig. 1C and E)*** to further simplify & speed up sample preparation.

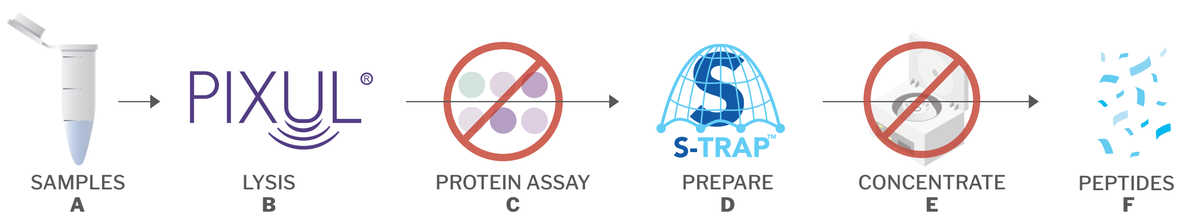


Fig. 1 *Streamlined workflow eliminates steps and variability*

WORKFLOW SIMPLIFICATION

S-Trap™ plates and columns efficiently capture proteins while removing vexatious contaminants like buffers, salts, reducing and capping reagents, detergents, polymers and small molecules. These impurities can interfere with protein assays, digestion, and MS analysis.

S-Trap™ Turbo improves upon the original design with polymeric materials featuring over 300 times greater derivatization density ***(Fig. 2A to B)***. This enhances binding efficiency, reduces the volume of captured matrix required, and yields small elution volumes which eliminates the need for additional concentration steps. Elutions are compatible with immediate direct LC-MS injection.

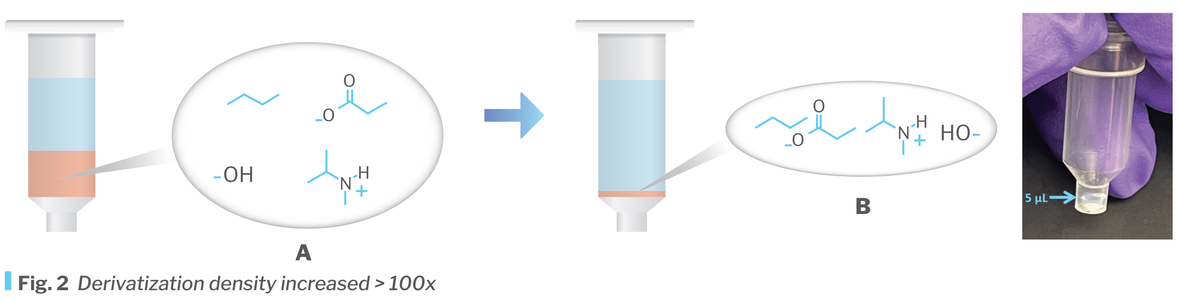


Fig. 2 *Derivatization density increased > 100x*

BCA-No-More™ integrates protein quantification directly into the same S-Trap™ plate used for downstream processing, eliminating both the need for a separate assay and sample loss. This time-saving method accommodates a wide range of lysis buffers that would otherwise disrupt sample preparation. Proteins loaded onto S-Traps™ are initially captured at the top of the column ***(Fig. 3)***, becoming more concentrated as the uppermost affinity sites fill. This surface-concentration of proteins with intrinsically fluorescent tryptophan, tyrosine, and phenylalanine residues ***(A)*** allows fluorescent protein quantification through top excitation ***(B)*** and detection of top emission ***(C)*** more protein results in more intense emission.

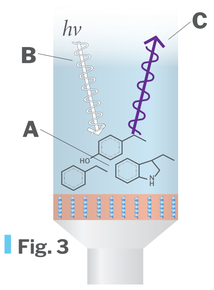


Fig. 3

METHODS

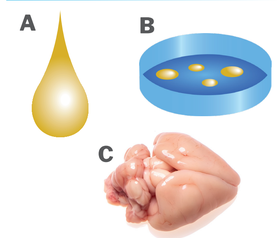


Fig. 4 *Samples tested*

New **S-Trap™ Turbo** followed standard protocols for lysis, reduction, alkylation, denaturation, binding, washing, and tryptic digestion. Various sample hydrophobicities were tested including: serum *(most hydrophilic, A)*, HeLa or HEK cell lysates *(varying hydrophobicities, B)*, and rabbit brain acetone powder *(most hydrophobic, C)*. High-resolution mass spectrometry *(Agilent QTOF 546/6550, Thermo Orbi-class, or Bruker timsTOF Pro)* was used to analyze samples, and BCA ***(Fig. 4A)*** and fluorescent assays ***(Fig. 4B)*** assessed sample yield and quality. Pierce 2 mg/mL BSA served as the protein standard.

The optimal position *(2100 µm)* for protein concentration quantification via fluorescence on the S-Trap™ 96-well plate was determined using a Tecan Spark plate reader. Protein fluorescence was measured in wet and dry states, with excitation between 269 and 280 nm and emission between 325 and 475 nm in top-read mode.

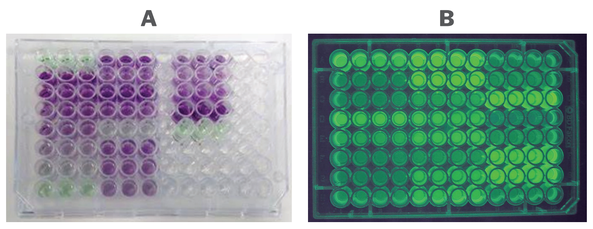


Fig. 5

RESULTS

After digestion, over 80% of the total peptide amount was recovered in the first elution fraction, equivalent to roughly 60% of the initially applied protein mass ***(Fig. 6)***. The columns exhibited high binding efficiency, capturing approximately 90% of applied protein.

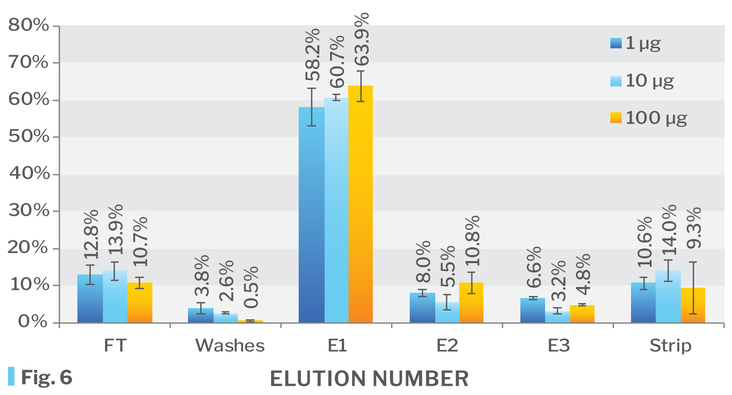


Fig. 6

RESULTS (cont.)

Recovery — S-Trap™ Turbo Column: Recovery remained steady regardless of increases in elution volume from 5 µL to 10 µL or 25 µL ***(Fig. 7)*** or protein amounts from 1 µg to 100 µg ***(Fig. 6 and 8)***; this was measured on S-Trap™ Turbo Micros.

In terms of digestion completeness, peptide yield, identification numbers and detected proteins, S-Trap™ Turbos yielded statistically identical results compared to standard S-Traps™.

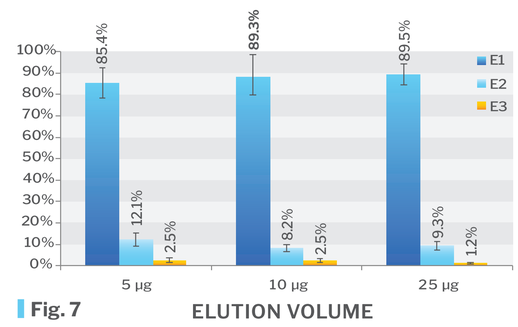


Fig. 7

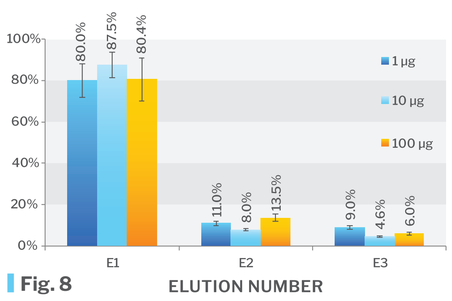


Fig. 8

Second and third elutions yielded an additional ~10% of the total applied protein ***(Fig. 8)***. Consistent elutions as low as 5 µL were achieved ***(Fig. 7)***.

Recovery was consistent across sample types, volumes did not necessitate speed-vaccing and S-Trap™ Turbo elutions could be directly loaded onto an auto sampler with or without acidification.

Fluorescent conditions: Optimal excitation and emission were found to be 277 nm and 350 nm, respectively, except for very low protein amounts where 410 nm emission detection was found to yield the best S/N. BCA-No-More™ quantification exhibited a linear response up to approximately 100 µg per well. Beyond this, the response decreased as fluorescent moieties bound deeper within the UV-opaque trapping matrix, forming a curve with a "hook" ***(Fig. 9)***.

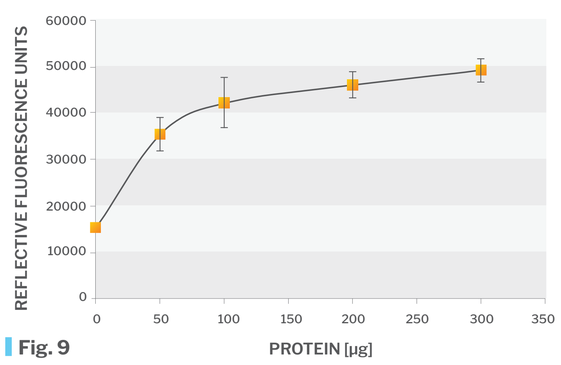


Fig. 9

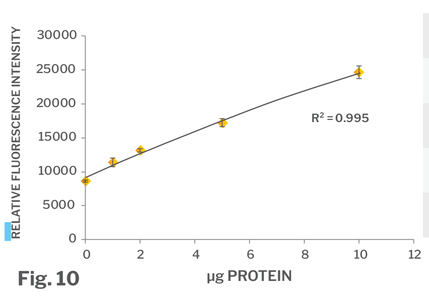
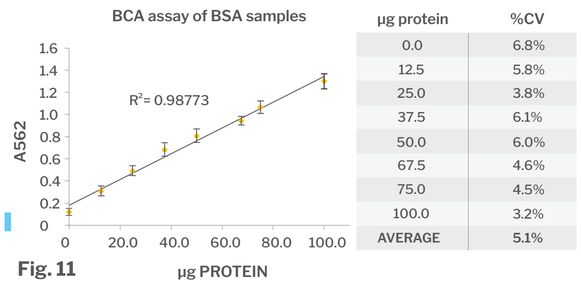


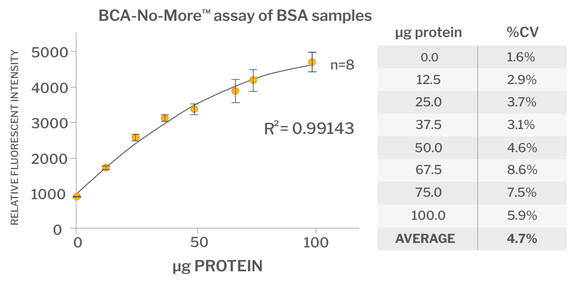
Fig. 10

µg PROTEIN	%CV
0	1.7%
1	5.4%
2	1.8%
5	3.5%
10	3.8%
AVERAGE	3.3%

Quantification: Protein quantification showed strong correlation and similar accuracy between traditional BCA assays ***(CV ~5%, R^2 ≈ 0.99, Fig. 11A)*** and on-plate BCA-No-More™ ***(CV ~3% – 5%, R^2 ≈ 0.99, Fig. 11B)***. This method enabled detection of protein concentrations as low as 1 µg ***(Fig. 10)***, an amount for commonly encountered in samples like in laser capture microdissection, and an amount not accessible via colorimetric assays. Absorbative losses of such limited amounts were mitigated by handling proteins in 5% SDS during all steps.



µg protein	%CV
0.0	6.8%
12.5	5.8%
25.0	3.8%
37.5	6.1%
50.0	6.0%
67.5	4.6%
75.0	4.5%
100.0	3.2%
AVERAGE	5.1%



µg protein	%CV
0.0	1.6%
12.5	2.9%
25.0	3.7%
37.5	3.1%
50.0	4.6%
67.5	8.6%
75.0	7.5%
100.0	5.9%
AVERAGE	4.7%

Fig. 11

CONCLUSION

A streamlined, high-throughput workflow. This combined approach promises significant reduction in the time and money from sample receipt to analysis readiness, crucial aspects for advancing high-throughput proteomic research and applications.

The combined S-Trap™ Turbo and BCA-No-More™ solution offers:

- A single, streamlined workflow: one universal protocol for all sample types.
- Higher efficiency: no more drying steps; known, consistent processing times.
- Greater recovery: minimal absorbative losses with zero loss to quantification.
- Integrated quantification as low as 1 µg with no separate assay needed!
- "Elute-and-shoot" peptides: ready for analysis in ≥20 µl for mini plates.
- Maximal simplification: less handling means fewer errors and repeats.
- Cost and time-savings: fewer steps, faster results, fewer consumables.

Old Approach	S-Trap™ Turbo + BCA-No-More™
Technician: $90,700 / YR[1]	One time automation cost
$200 - $200 per kit	No extra kit required
Minimum Sample Amount (LLOD)	No need to sacrifice precious sample
Must avoid high concentrations of reducing agents	Suitable for harsh lysis buffers including those for FFPE

[1]*Salary at 62.5th percentile in Bay Area, Boston, & Research Triangle*

Fig. 12

[1]*ProtiFi LLC, Fairport, NY, United States;* [2]*University of Leeds, Leeds, United Kingdom*

**ProtiFi technologies are patent and patent-pending.*

Conflict of Interest: The authors are the developers, inventors, and/or owners in or of ProtiFi LLC. Notwithstanding, we present these results as scientists.