S-Trap turbo: from samples to analysis in record time

Sandra Wilson[1]; Darryl J.C. Pappin[2]; Alexandre Zougman[3]; John Wilson[1]

[1]ProtiFi, LLC, Farmingdale, NY; [2]Cold Spring Harbor laboratory, Cold Spring Harbor, NY; [3]University of Leeds, Leeds, United Kingdom

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I. Introduction

With the ability to handle extremely diverse sample types at varied operator skill levels and without the need for protocol modification, the S-Trap sample preparation system has found widespread adoption in the proteomics community

However, recent advances in data acquisition throughput, in some cases now only minutes per sample, necessitate concomitant advances in bottom-up sample preparation

The elimination of any and all extraneous elements in a sample preparation workflow increases both throughput and robustness. One of the more tedious (and often time-variable) steps in proteomics sample processing is post-elution sample dry-down

Here we present the new S-Trap turbo: S-Traps that yield minimal elution volumes of highly concentrated peptides suited for immediate analysis by injection on LC-MS or spotting on MALDI.

II. Methods

Novel snap-cap S-Trap turbo micro columns were constructed via plastic injection moulding.

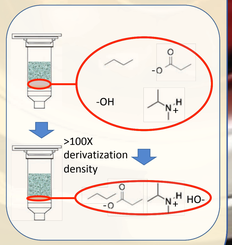
The columns incorporated newly developed, low-binding polymeric traps densely derivatized with novel surface modifications hundreds of times more dense than in traditional S-Traps.

The standard S-Trap protocol steps of lysis, reduction and alkylation, denaturation, binding, washing and tryptic digestion were performed both for standard and turbo S-Traps

Samples were analyzed by LC-MS on an Agilent QTOF (6546 and/or 6550), Orbi-class instrument or Bruker timsTOF

Sample yield was compared and quantified using BCA and/or fluorescent assays.

Sample quality was compared by peptide and protein identification rate and reproducibility of quantifications.



III. Small Elution volume

Turbo traps were compared to traditional S-Traps using three samples of highly varied hydrophobicity:

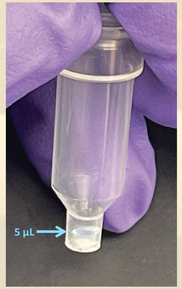
- Serum (most hydrophilic)
- HeLa or HEK cell lysate (both hydrophilic and hydrophobic)
- Rabbit brain acetone powder (most hydrophobic)

Between < 1 µg to 100 µg of protein was processed

S-Trap turbo elutions as low as 5 µL were found to be reproducible and similar or equivalent to standard S-Trap digestions as judged by:

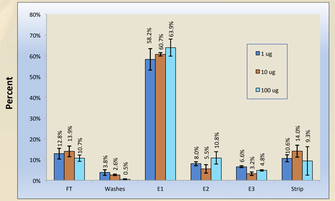
- completeness of digestion
- peptide yield and identifications
- numbers of identified peptides and proteins

S-Trap turbo elutions could be immediately loaded onto an autosampler with or without acidification



IV. Recovery as a function amount of input material

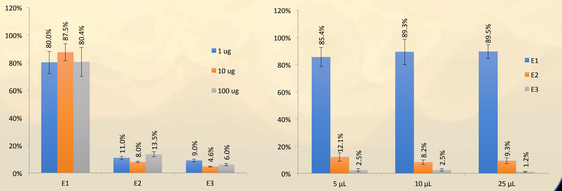
From 1 to 100 µg of protein was applied to beta S-Trap Turbos. Three elutions were performed: the initial 5 µL digestion volume, 10 µL then 20 µL (see next panel). ~90% of sample was bound by S-Trap Turbos. Approximately 60% of the total applied was recovered in the first elution. There was not a significant change in the behavior of the columns as a function of protein loading amount.



Key: FT = Flow Through, E = Elutions 1 through 3, Strip = SDS lysis buffer plus heat

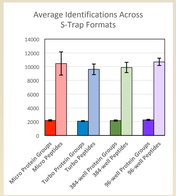
V. Recovery with small volume

Greater than or equal to 80% of the total digested, processed peptides was eluted in the first elution fraction; this represents recovery of approximately 60% of the total applied protein in this one fraction. This proportion was not significantly changed either by a change in the amount of loaded protein nor by increasing the elution volume from 5 µL to 25 µL. A second and third elution ultimately recover an additional ~10% of the total applied protein, a proportion that does not necessarily warrant speed-vacing.

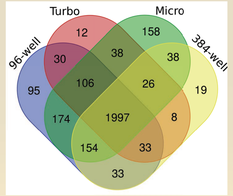


VI. Equivalence across S-Trap platforms

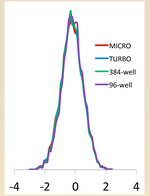
As measured by identification rates, rates of missed cleavage and GRAVY distributions, the different S-Trap format are statistically indistinguishable.



Identifications



Venn diagram of overlap



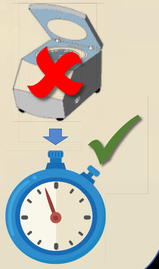
Peptide GRAVY distribution

VII. Time savings over standard protocols

By offering an "elute and shoot" solution, the S-Trap turbo affords a further increase in the efficiency of proteomics sample processing.

The speed at which samples can be concentrated depends on many factors including the number of samples and their volume, the kind of solvent (percent organic content), the temperature, the efficiency of solvent removal or trapping, which drives the process, and the extent of vacuum, which itself is a function of the pump and the factors above. Thus, speed-vacing times can range from minutes to many, many hours. Additionally, researchers frequently encounter the situation in which a single stubborn sample refuses to dry down, sometimes even in the case of identically aliquoted tubes, necessitating additional time, temperature or both, and adding additional uncertainty to experimental planning.*

The S-Trap turbo directly solves these issues by yielding clean peptides, ready to inject, with the same efficiency as other formats that require dry down.



*if you understand the reason for this, please email explanations or hypotheses to john@protifi.com. At present, modulation by unequal amounts of plasticizers and mould release agents (etc.) in different tubes of the ability of the solution to evaporate is our leading theory see e.g. Kim DO, Rokoni A, Kaneelil P, Cal C, Han LH, Sun Y. Role of surfactant in evaporation and deposition of biosolvent biopolymer droplets. Langmuir. 2019 Sep 9;35(39):12773-81. Alternatively, different effects of interaction between the tube surface and evaporating liquid.

VIII. Conclusions

No significant loss of hydrophobic peptides between the standard S-Trap protocol and S-Trap turbo was observed, an observation likely explained by the use of aqueous or mostly-aqueous buffer A to solubilize peptides (followed by hard centrifugation) and the traps having matched hydrophilicity.

Sample processing time on S-Trap turbos could be further reduced to mere minutes, through the use of sonication

S-Trap turbo allows proteomics researchers to go from complex samples to ready-to-inject peptides in record time, with minimal steps and equipment

We anticipate they will enable the broader use of proteomics techniques