S-Trap sample processing for™ rapid (< 1 hr) and unbiased digestion of submicrogram to milligram scales

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1) Overview

- S-Trap™ technology* enables rapid, reproducible and universal sample processing for bottom-up proteomics. It combines strong SDS-based protein solubilization with integrated sample cleanup and rapid reactor-type protein digestion. S-Trap™ sample processing is simple, can be performed in any lab with standard equipment and works with your choice of protease.
- Total S-Trap™ sample processing is very fast: removal of SDS and contaminants (PEG, detergents, salts, glycerol…) takes only few minutes, and complete digestion can be done in 30 min. This speed can assist to observe and analyze labile biological events such as PTMs.
- S-Trap™ technology is now available in a 96-well plate format for high throughput (<1 minute per sample average). Midi scales are available for large scale (2 – 10 mg) useful for pre-enrichment, e.g. in phosphopeptide or SISCAPA/immunoaffinity analysis. Nano, micro, mini S-Trap™ formats allow processing from sub-microgram to 300 µg with low losses.
- S-Trap™ processing is suitable for all sample types including cells, tissues, membranes and serum. Proteins of all solubilities are equally sampled: cytosolic and membrane proteins are identically solubilized and processed.
- Proteases and phosphatases are inactivated in standard S-Trap™ processing (no inhibitors needed) and samples are “autofiltered.”

2) Introduction

The Suspension-Trapping™ or S-Trap™ method is based on SDS-mediated protein extraction (SDS ≤ 15%) which solubilizes most proteins. Detergent is swiftly removed, yielding an ultrafine, detergent-free protein particulate, which is retained and cleaned within submicron pores of a protein trap (the S-Trap™). Proteases are introduced into the pores where tight physically confinement greatly enhances protease-substrate interaction and thus proteolytic activity to enable rapid (< 1 hr), reactor-type digestion. Robust performance of the smaller scale S-Trap™ with all protein types, including membrane proteins, led to desire for high-capacity units able to process multiple milligrams: especially in the analysis of post-translational modifications (PTMs), enrichment of the desired component(s) often requires milligram-scales of input protein. We present a new, large-capacity midi S-Trap™ which is easily manipulated by centrifugation or vacuum manifold. Introduction of protein into the trap (SDS depletion, wash and protease addition) requires minutes. After a one-hour digest at 47 °C, peptides are eluted and ready for downstream processing.

3) S-Trap™ sample processing

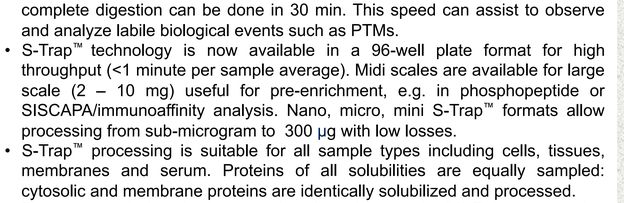


Fig. 1: Steps of S-Trap™ sample processing.

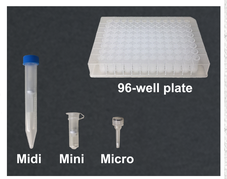


Fig. 2: Available formats of S-Traps™. Micros handle < 100 µg, minis and the 96-well plate 100 – 300 µg and midis > 300 µg.

***S-Trap™ technology is patent-pending.**

4) Materials and methods

96-well plate S-Traps™, large-scale midi units as well as small-scale S-Trap™ micro and mini units were manufactured by ProtiFi LLC (www.protifi.com, Huntington, NY). Cells were grown in standard cell culture conditions. Acetone powders from rabbit muscle, thymus and brain were purchased from Pel-Freez Biologics. Chemicals and solvents were reagent (or better) or HPLC grade, respectively. Samples were lysed SDS lysis/extraction buffer (5% SDS in 50 mM TRIS-HCl, pH 7.6) at room temperature (RT). Lysate viscosity reduction and DNA sheering was accomplished by brief probe sonication. Samples were reduced in the SDS lysis buffer with dithiothreitol (DTT) added from a 1 M stock a final concentration of 20 mM. The lysate was heated to 95 °C for 5 min, cooled to RT alkylated with addition of iodoacetamide (IAA) in the dark, clarified by centrifugation at 14,000 g for 10 min and assayed for protein concentration (BCA assay or tryptophan fluorescence). S-Trap™ midi units were used as follows: ~0.5 – 1 mL of SDS lysate containing around 2 – 10 mg protein was acidified by addition of phosphoric acid to a final concentration of 1.2%. To this was added S-Trap™ buffer (90% MeOH, 100 mM Tris-HCl pH 7.1) at ~6x the volume of this acidified lysate, instantly forming a fine protein precipitate. Protein was trapped by vacuum manifold or centrifugation. Protein and protein trap were washed with ≥1.2 mL of S-Trap™ buffer. Flow through (FT) and wash were typically collected and combined for later verification of protein capture. To the washed trap, trypsin or other protease contained in 0.5 mL of 40 mM ammonium bicarbonate (0.16 mg/mL) was added at 1:25 to 1:40 wt:wt and brought into the trap by gentle centrifugation. Digestion proceeded at 47 °C for 0.5 - 3 hours, after which time the digest was collected by centrifugation. Peptides were further eluted first with 600 µL of 40 mM ammonium bicarbonate and second with 600 µL of 0.2% aqueous formic acid. Sometimes a final organic elution of 600 µL 0.2% formic acid in 50% aqueous acetonitrile was added; this is obligatory for C18 versions. Elutions were combined and concentrated for analysis. Any remaining protein in the trap was stripped by passing 500 µL of SDS lysis buffer through the unit; this strip fraction was collected and analyzed to verify full recovery. MS runs were on Orbitrap-class machines using standard methods.

5) Unbiased protein processing

S-Trap™ sample processing begins by bringing protein into solution with high concentrations of SDS, typically around 5% - 8%. SDS is highly effective at solubilizing even poorly soluble proteins such as membrane proteins or fibrous materials like chromatin, muscle, etc. Under these conditions, proteases and phosphatases are inactivated, reducing or eliminating the need for inhibitors and thus reducing the cost of sample processing.

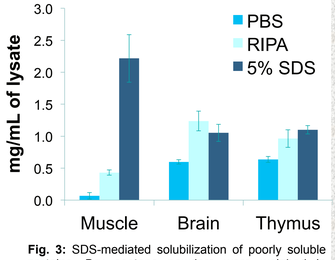


Fig. 3: SDS-mediated solubilization of poorly soluble proteins. Dry acetone powders were weighed in triplicate. Various lysis buffers were added, samples sonicated, incubated on an end-over-end rotator and assayed for protein concentration.

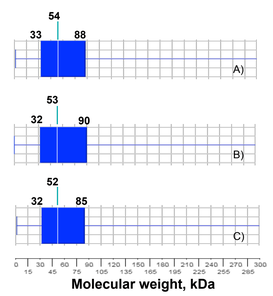


Fig. 4: Molecular weight distributions of proteins identified from HeLa cells by three methods show no significant differences. Box and whisker plots. A) FASP, 10,237 proteins[2] B) RIPA extraction, TCA, and urea/SDS digest, 12,593 proteins[3] C) S-Trap™ (2 SCX fractions), 4,299 proteins[1].

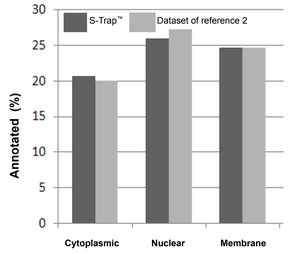


Fig. 5: Similar proportions of proteins from various cell compartments of HeLa cells are identified with S-Trap™ processing[1] and the complete proteome[2].

5) Unbiased protein processing continued

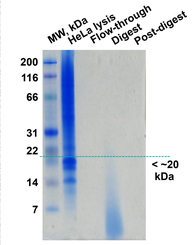


Fig. 6: S-Trap™ sample processing with rapid SDS removal and protein precipitation captures the full range of protein sizes including lower molecular weight proteins. Compare also Fig. 4.

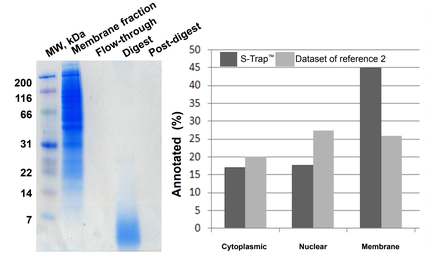


Fig. 7a (left): S-Trap™ processing of Triton X-114 extracted membrane proteins were solubilized in 15% SDS and digested for 1 hr with trypsin.[1] **Fig. 7b** (right): the fraction of identified membrane proteins in the membrane protein extraction increased from 25% (Fig. 4 above) to 45%.[1]

6) Speed of digestion

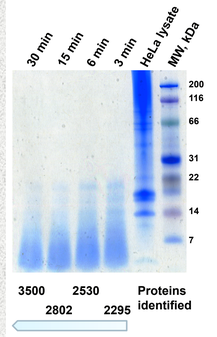
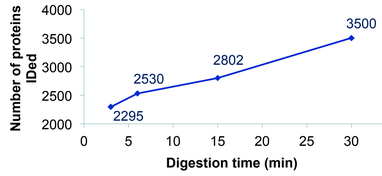


Fig. 8: Reactor-like digestion is rapid, allowing efficient protein ID in as little as 5 min; tryptic digestion is commonly done for 45 min at 47 °C[1]. Length of time may be sample- and enzyme dependent; for serum, the most reproducible digestion at multi-milligram scale was 1 hr.



7) Reproducibility of S-Trap™ digestion

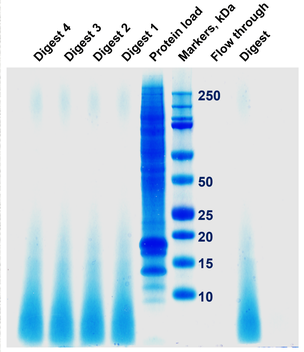
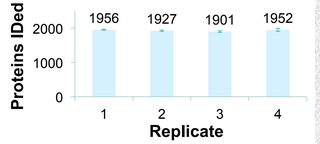


Fig. 9: S-Trap™ protein processing enables highly reproducible sample processing. PC3 cell lysate dissolved in 5% SDS was digested in five aliquots in S-Trap midi units with trypsin, 47 °C, 1 hr (see left). Proteins were identified in triplicate per digest on an LTQ-Orbitrap platform (90-min 1D LC-MS runs). The average number of protein identifications per digest was highly similar (see below)



8) Completeness of digestion

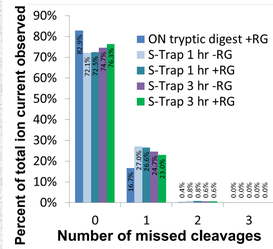


Fig. 10: S-Trap digests had similar total intensities of observation of fully tryptic peptides with 95.6% (light blue) 96.3% (teal), 97.9% (purple) and 98.1% (green) of the total current compared to a control overnight tryptic digest after normalization to total counts. (These are percentages of ion current for peptides with no missed cleavages only and are different than percent of total at left.) Addition of RapiGest (Waters) and also an additional 3 hr incubation at 37 C with additional trypsin at 1:100 post S-Trap elution decreased the missed cleavage rate. Without normalization, total recovery of 1 hr S-Trap processing was higher than the control; with an additional 3 hr incubation, it was lower.

9) S-Trap™ midi use in large-scale sample prep: serum

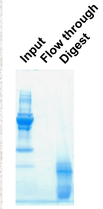
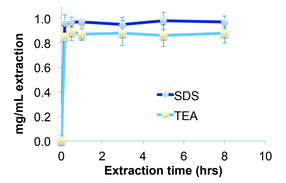


Fig. 11: Digestion of serum and plasma can be difficult due to high concentrations of disulfide-linked proteins, trypsin inhibitors and endogenous antibodies. These properties typically necessities the use of large concentrations of urea (8 M), which is then diluted prior to digestion and cleaned by reverse phase techniques. S-Trap™ protocols easily process serum without change to. At left, 2.5 mg of mouse serum was digested for 1 hr with trypsin at 47 °C. No precipitation was noted during or after reduction or digestion. No protein break-through was detected in the flow through. Similar results were obtained for SDS lysates from tissue acetone powders.

10) Extraction of dried blood spots

Fig. 12: Dried blood spots (Noviplex™ Plasma Prep Cards with the red blood cell fraction removed) were extracted either with triethylammonium bicarbonate (pH 7.4, 50 mM) containing or lacking 5% SDS. Extraction kinetics were rapid and SDS consistently extracted more protein.



11) Conclusions

- **S-Traps™ provide a universal digest solution:**
  - Unbiased, fast, easy, robust and reproducible
  - Sub-microgram to milligram scale; 96-well format for automation
  - Use your choice of protease(s).
- **Strong SDS-based protein solubilization allows all sample types:**
  - Cells, tissues, membranes, biological fluids, DBS...
- **S-Trap™ use automatically integrates sample cleanup.**
  - Removes PEG, detergents, salts, urea, glycerol…

13) References

1. Zougman, A., Selby, P.J. and Banks, R.E., 2014. Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis. *Proteomics*, *14*(9), pp.1006-1000.
2. Nagaraj, N., Wisniewski, J.R., Geiger, T., Cox, J., Kircher, M., Kelso, J., Pääbo, S. and Mann, M., 2011. Deep proteome and transcriptome mapping of a human cancer cell line. *Molecular systems biology*, *7*(1).
3. Guo, X., Trudgian, D.C., Lemoff, A., Yadavalli, S. and Mirzaei, H., 2014. Confetti: a multiprotease map of the HeLa proteome for comprehensive proteomics. *Molecular & Cellular Proteomics*, *13*(6), pp. 1573-1584.