Sample preparation to match analytical advances: 384-well S-Trap plates

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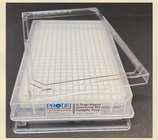
I. Introduction

Recent advances in analytical proteomics throughput, in some cases now requiring only minutes per sample for identification and quantification, necessitate concomitant progress in bottom-up sample preparation workflows

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 proteomics analyses

To date, S-Traps have been available as both spin columns of varying capacities and in 96-well plate format

To keep pace with advances in detection including ever-increasing throughput and single-cell analyses, we developed and present the new S-Trap 384-well plate, suited for protein loads from single cells and sub µg quantities to 100 µg.



II. Methods

384-well S-Traps were manufactured to match the performance of S-Trap micros

HeLa cells were grown according to standard tissue culture techniques.

Using this sample, replicate sample preparations were performed on 384-well plates; S-Trap micro columns, with 96-well plates used as a baseline

The standard steps of lysis, reduction, alkylation, denaturation, binding, washing and digestion were performed as per standard protocol

Well-to-well and plate-to-plate variation were compared based on contaminant removal, extent of recovery, extent of digestion and detection via analysis by LC-MS on an Agilent QTOF 6546, Bruker timsTOF Pro and Thermo

Samples

Lysis

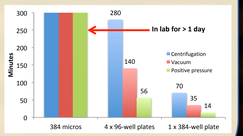
Protein assay

Prepare: 384- and 96- well, micro columns

LC-MS

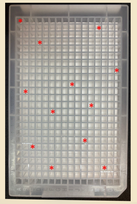
III. Relative processing times

While the 96-well S-Trap plate affords a significant increase in sample throughput, all S-Trap formats including the 384-well plate require the following sequence of events: 1) protein with binding buffer is applied; 2) this protein is washed 3X; 3) the protein is digested; and 4) the protein is eluted, typically in 3 elutions. Each of these 7 steps require that solution pass through the plate, and each thus imposes a time demand. In centrifugation, spin up and down times can be significant (depending on the equipment) and a single step might require 10 min. For the A200 with positive pressure, this is shortened; we'll call it 2 min/step average. Vacuum manifolds are notoriously variable depending on the samples and if they go dry before others; 5 min is assumed. Under these assumptions, the graph at right shows the efficiency of a 384-well plate.



IV. Reproducibility across wells

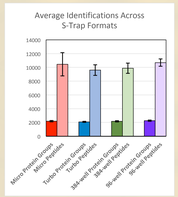
Identical replicates of serum were processed on a 384-well plate. 12 were randomly chosen, identified and quantified on an Agilent 6546. Pearson's correlations of quantifications were between 1 and 0.98, which was observed once.



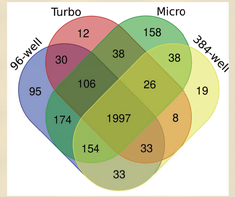
1.00	0.99	0.99	1.00	0.99	1.00	0.99	0.99	1.00	1.00	1.00	0.99
0.99	**1.00**	0.99	0.99	0.99	0.99	0.99	1.00	1.00	0.99	0.99	1.00
0.99	0.99	**1.00**	0.99	1.00	0.99	0.99	0.99	0.99	1.00	0.99	1.00
1.00	0.99	0.99	**1.00**	0.99	1.00	1.00	0.99	0.99	0.99	1.00	0.99
0.99	0.99	1.00	0.99	**1.00**	0.99	0.99	0.99	1.00	0.99	0.99	1.00
1.00	0.99	0.99	1.00	0.99	**1.00**	0.99	0.99	1.00	1.00	0.98	1.00
0.99	0.99	0.99	1.00	0.99	0.99	**1.00**	1.00	1.00	0.99	1.00	0.99
0.99	1.00	0.99	0.99	0.99	0.99	1.00	**1.00**	0.99	1.00	1.00	1.00
1.00	1.00	0.99	0.99	1.00	1.00	1.00	0.99	**1.00**	0.99	0.99	1.00
1.00	0.99	1.00	0.99	0.99	1.00	0.99	1.00	0.99	**1.00**	0.99	1.00
1.00	0.99	0.99	1.00	0.99	0.98	1.00	1.00	0.99	0.99	**1.00**	0.99
0.99	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00	0.99	**1.00**

V. Comparative performance to other formats

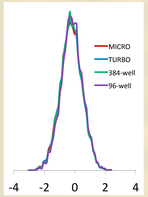
As measured by identification rates, rates of missed cleavage and GRAVY distributions, the different S-Trap format are statistically indistinguishable.



Identifications



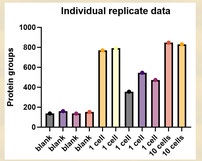
Venn diagram of overlap

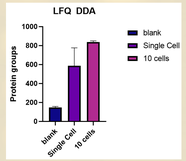


Peptide GRAVY distribution

VI. Single cell applicability

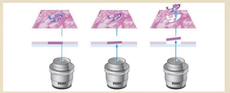
Individual NCI-H358 cells were sorted by FACS into 96 well plates. Cells were solubilized in 12 uL of S-Trap lysis buffer. For 10 cells, 12 uL of S-Trap lysis buffer was used to resolubilize 10 separate wells by transfer of liquid and repeat pipetting to resuspend each individual cell. The final 12 uL was processed identically to the single cells. Reduction and alkylation was not performed. The high recovery S-Trap protocol was followed using S-Trap 384 well plates. Peptides were eluted and concentrated by speedvac. LFQ runs were performed using a TIMSTOF Flex instrument and EasyNLC LC system operating at 200nL/min using a 2 hour gradient in DDA mode. An IonOpticks Aurora 25cm column was used for the separation

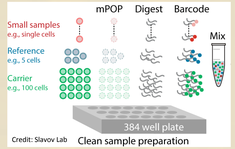




VII. Future Directions

- Automated high throughput
- Clinical applications
- Laser capture microdissection
- Single cells





VIII. Conclusions

- 384-well plates provide for robust high throughput automated sample preparation
- The plate is suited for scales from single cell to 100 µg
- The wells are equivalent to micro columns
- Sample prep now averages < 10 sec per sample
- This level of throughput provides the level of throughput needed for clinical applications