White Paper

RAININ
Pipetting 360°

High Capacity Desalting

PureSpeed C18 Tips

Reverse phase adsorption is a technique often used to desalt and concentrate biomolecules such as peptides and proteins. Used extensively for sample preparation, a hydrophobic stationary phase comprising alkylsilane-modified silica particles is used to retain hydrophobic molecules.

Hydrophilic molecules or salts are not retained by the stationary phase so these species are removed from the sample. After washing steps to remove residual hydrophilic molecules and salts, the hydrophobic molecules of interest are eluted (recovered) from the stationary phase by a non-polar solvent. The usually volatile non-polar solvent evaporates from the final sample and the pellet can be dissolved in aqueous buffers.

The most common type of reverse phase silica, C_{18}, can be immobilized at the distal end of a pipette tip to facilitate sample preparation involving peptides prior to mass spectrometry. Currently available C_{18} tips exhibit significant utility in mass spectrometry, but also suffer from a number of disadvantages:

- Not all C_{18} tips provide comparable levels of percent sequence coverage in mass fingerprinting experiments.
- Many C_{18} tips are limited in terms of the capacity for peptides they can provide.
- While commercially available tips can be used with universal pipettes, there is no ergonomic pipetting solution currently available.
- Although repetitive and tedious, the desalting protocol for C_{18} purification can only be automated using expensive liquid handing robots.

Rainin Instrument, a subsidiary of METTLER TOLEDO, has developed PureSpeed C18 desalting tips for the purification of peptides from tryptic digest as a prelude to tandem mass spectrometry.

METTLER TOLEDO

PureSpeed C18 Desalting tips have a number of benefits compared to existing desalting tip products:

- PureSpeed C18 Desalting tips provide adequate capacity for processing approximately 10 µg of peptide per tip, greater than products currently on the market.
- These tips provide excellent signal-to-noise ratio and percent sequence coverage in tandem mass spectrometry analysis of protein digests
- They are available in universal and ergonomic LTS formats
- PureSpeed software on Rainin E4 XLS pipettes enables semi-automated desalting of up to 12 samples in parallel.

This document describes the performance of PureSpeed C18 Desalting tips in proteomics workflows.

Experimental Results

Rainin has conducted experiments to define PureSpeed C18 Desalting tip performance. First, the overall capacity of the tips was determined. In the experiment described below, an increasing amount of peptide was bound to PureSpeed C18 Desalting tips prior to eluting and quantifying the peptide in the eluate. In this case, the tips were able to retain greater than 10 µg of peptide when saturated (Figure 1). This amount of peptide is greater than reported capacities for a number of C18 desalting tips from other manufacturers.

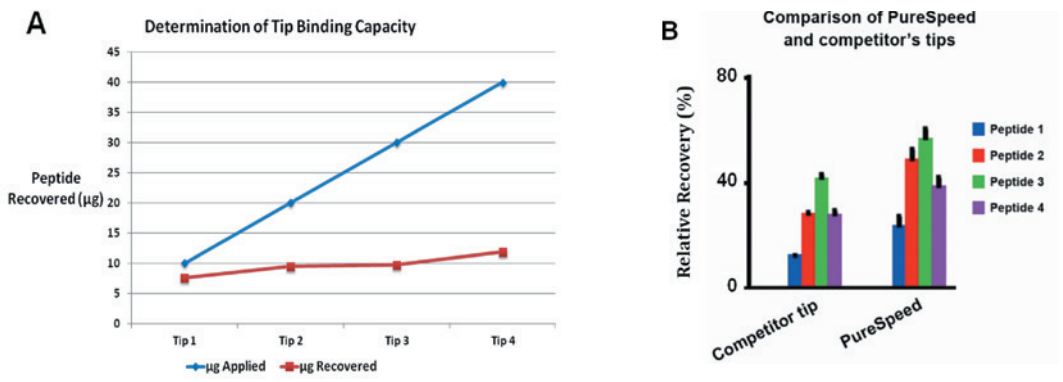

Figure 1. PureSpeed C18 Desalting Tip Capacity

Panel A shows the capacity of PureSpeed C18 Desalting tips. The capacity was ascertained by binding increasing amounts (10 µg, 20 µg, 30 µg, or 40 µg) of peptide LLLLDFR (P4 below) to properly conditioned PureSpeed C18 Desalting tips. After eluting peptides from the tips, the volume was reduced to ~2 µL using a centrifugal evaporator. The sample volume was then adjusted with 0.1% TFA, 2% acetonitrile, 98% water to 1 µg/µL (assuming 100% recovery, e.g., 10 µL for 10 µg Stock etc.) before injecting 4 µL on a C18 HPLC column and comparing the recovery relative to injection of 4 µL of 1 µg/µL solution of LLLLDFR.

Panel B shows the recovery when 2 µg each of four peptides are applied to PureSpeed and competitor desalting tips, then washed, eluted, and quantified by HPLC. As it can be seen, PureSpeed C18 Desalting Tips are able to recover more peptide versus competitor desalting tips.
The peptide sequences were:
P1 – NH2-LGGGGGGDFR-COOH
P2 – NH2-LLGGGGDFR-COOH
P3 – NH2-LLLGGDFR-COOH
P4 – NH2-LLLLDFR-COOH

When a mixture of four peptides were loaded and purified with PureSpeed C18 Desalting tips, or a leading brand from another manufacturer, it was observed that PureSpeed C18 Desalting tips consistently provided greater recovery of each peptide.

Next, the percent sequence coverage of PureSpeed C18 Desalting tips was tested, and tryptic digests were prepared for model proteins. After desalting on PureSpeed C18 Desalting tips, the peptides were analyzed by tandem mass spectrometery (LC-MS/MS). For each protein, the ProteinPilot™ percent coverage was determined (Figure 2).

Protein	PureSpeed C18	Competitor C18
Insulin	100.0%	56.9%
Cytochrome c	53.3%	52.4%
Apomyoglobin	51.9%	41.6%
β-Lactoglobulin	50.0%	50.0%
Carbonic anhydrase	47.3%	70.0%
Superoxide dismutase	32.2%	32.2%
Alcohol dehydrogenase	56.3%	56.3%
BSA	57.9%	61.7%

Figure 2. PureSpeed C18 Desalting Tip Sequence Coverage. 500 pmoles of each protein were dissolved in 50 mM Tris-HCl (pH 8), 8M urea, and 2 mM DTT and heated to accomplish protein denaturation. After heating and cooling, the protein was diluted with 50 mM $NaHCO_3$ plus 1 mM $CaCl_2$ in order to reduce the urea concentration to below 1 M. Trypsin was added in a 1 to 20 ratio (mass amount of trypsin versus mass amount of the protein) and the solutions were incubated at 37°C overnight. After terminating the trypsin reaction by reducing the pH to below 4 using 1 % TFA, the peptides were diluted to an estimated 200 fmoles/µL using 50 mM NH_4HCO_3 plus 1 M urea prior to desalting with PureSpeed C18 Desalting tips. After drying down the eluate with centrifugal evaporation, the sample volume was adjusted by adding buffer prior to loading onto an LC-MS/MS and collecting sequence coverage data.

In terms of sequence coverage for different proteins, PureSpeed C18 Desalting tips compared almost identically to desalting tips from a leading manufacturer used in the same workflow.

The performance of PureSpeed C18 Desalting tips was then tested in a peptide purification workflow (Figure 3). Here, a 2-D gel of E. coli lysate was run and individual protein spots were excised. The proteins within the gel spots were digested in situ with trypsin and then desalted using PureSpeed C18 Desalting tips or desalting tips from another manufacturer. The peptides were then analyzed via MALDI-TOF/TOF mass spectrometry and the number of peptides purified per spot determined. Additionally, the peptide percent sequence coverage per protein identified was ascertained. From each gel spot, PureSpeed C18 Desalting tips were able to enrich for a similar amount of peptides as compared to a competitor's desalting tips.

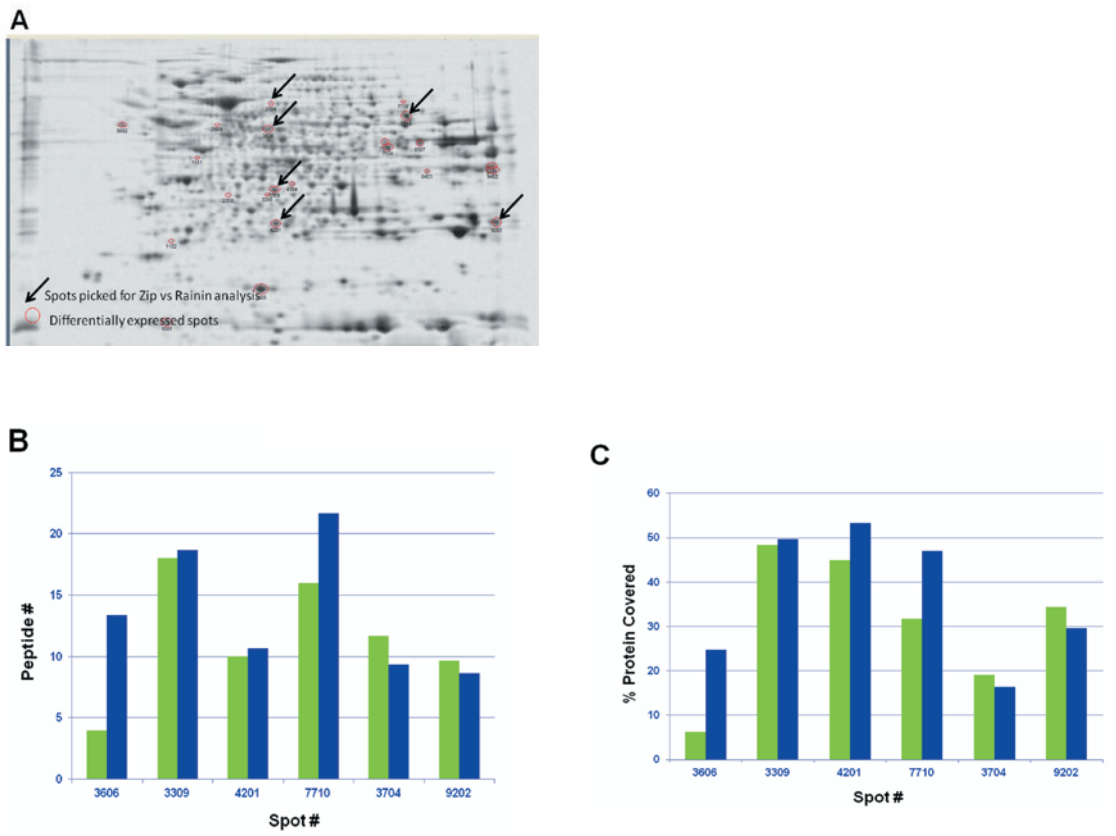

Figure 3. Performance of PureSpeed C18 Desalting Tips in a Proteomics Workflow – Peptide Purification.
Panel A shows a 2-D gel and the associated protein spots that were excised prior to digestion, desalting, and mass spectrometry. In the following panels B and C, competitor's tips are represented by green bars, while Rainin tips are represented by blue bars. Panel B shows the number of unique peptides determined for each gel spot. PureSpeed provides a comparable, yet unique peptide recovery profile. The number of peptides identified using PureSpeed versus the product from another manufacturer are roughly equivalent. Panel C shows the percent of protein sequence covered by the peptides purified with Rainin PureSpeed tips or other manufacturer tips. PureSpeed provides excellent sequence coverage.

We further investigated the use of PureSpeed C18 Desalting tips in an intact protein purification workflow by using the tips in the following protocol. Here, MS-1 cancer cells were lysed, and the lysate was fractionated using PureSpeed strong cation exchange tips and four salt fractions (50 mM, 100 mM, 200 mM, and 400 mM) were collected. After desalting each of the fractions using PureSpeed C18 Desalting Tips, the samples were digested with trypsin and subjected to mass spectrometry. LC-MALDI was carried out on an AB SCIEX 5800 TOF/TOF™ System and LC-MS/MS was carried out on a ThermoFisher LTQ-Orbitrap Velos™ using a 90 minute gradient for reverse phase separation. Collectively, the two techniques afforded a number of unique protein identifications (Figure 4).

In Figure 4 a number of different proteins were identified using PureSpeed C18 Desalting Tips. The data in Figures 3 and 4 illustrate that PureSpeed C18 can be employed in many different experimental workflows. In Figure 3, the utility of PureSpeed to purify peptides was demonstrated, while in Figure 4, PureSpeed was shown to be able to accommodate intact protein purification.

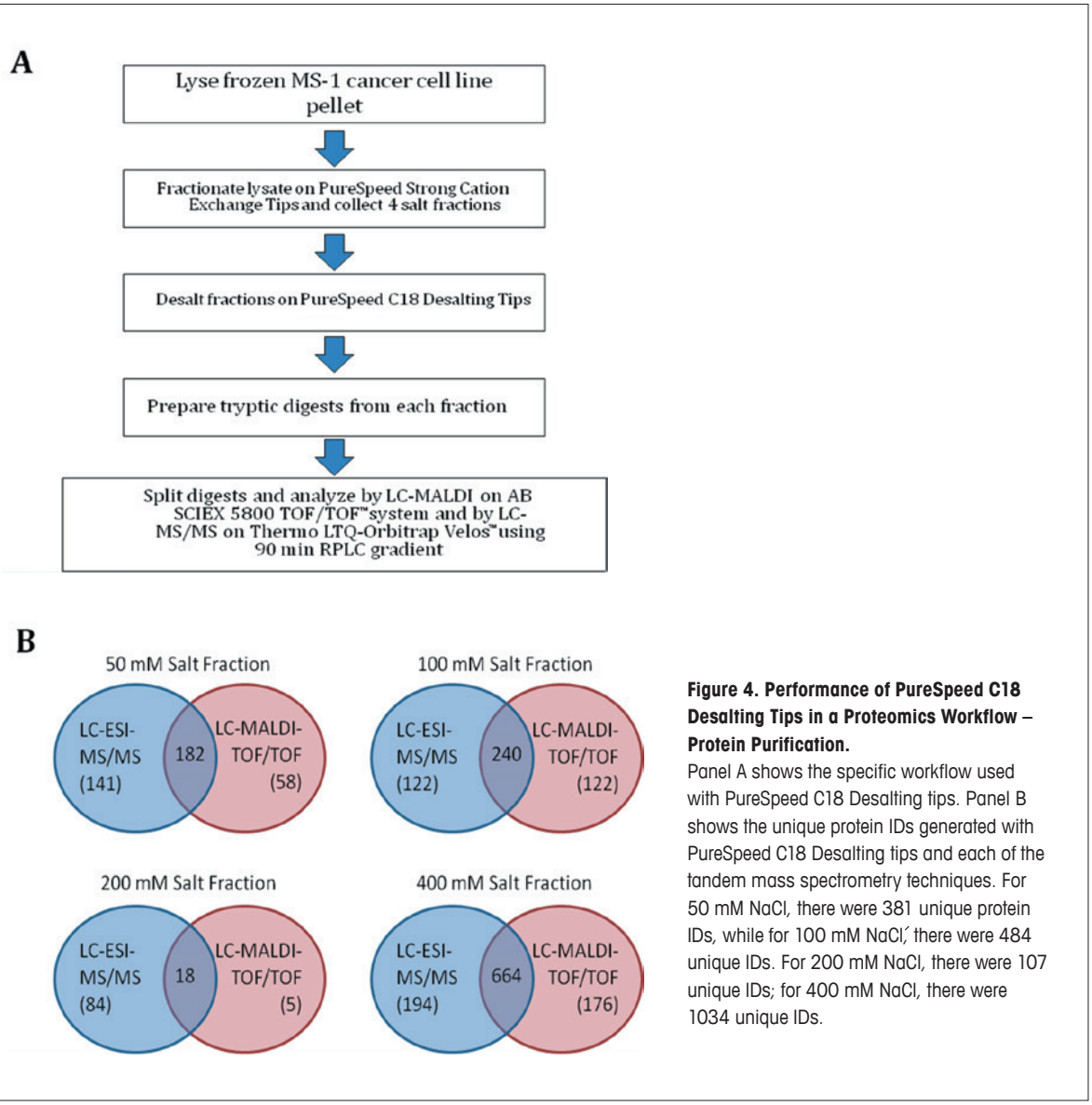

Figure 4. Performance of PureSpeed C18 Desalting Tips in a Proteomics Workflow – Protein Purification.
Panel A shows the specific workflow used with PureSpeed C18 Desalting tips. Panel B shows the unique protein IDs generated with PureSpeed C18 Desalting tips and each of the tandem mass spectrometry techniques. For 50 mM NaCl, there were 381 unique protein IDs, while for 100 mM NaCl, there were 484 unique IDs. For 200 mM NaCl, there were 107 unique IDs; for 400 mM NaCl, there were 1034 unique IDs.

Conclusions

The data above shows that PureSpeed C18 Desalting tips can effectively desalt peptides and proteins and be used to generate high-quality mass spectrometry data. In terms of the desalting protocol, the use of LTS pipettes and the PureSpeed mode enabled an easiier, more ergonomic workflow. LTS pipettes reduce tip loading and ejection forces for desalting workflows, while the PureSpeed program on the E4 XLS pipette allows for semi-automated multiple sample processing that minimizes user errors and ensures reproducibility. PureSpeed C18 Desalting tips perform extremely well in proteomics workflows.

As illustrated in the examples above, excellent percent sequence coverage was obtained from protein digests desalted on PureSpeed C18 Desalting tips and analyzed by tandem mass spectrometry. Furthermore, PureSpeed C18 was able to fractionate intact proteins from cell lysates prior to digestion. In terms of peptide yield, PureSpeed C18 Desalting tips are excellent, providing >10 µg of peptide capacity per tip.

PureSpeed C18 Desalting tips offer benefits to researchers carrying out proteomics workflows:

- Many options in terms of tip and pipette types
- A simple automated protocol
- Higher peptide binding capacity versus many products on the market
- Excellent sequence coverage in mass spectrometry fragment analysis
- The capability to bind whole proteins for mass spectrometry analysis

Figure 5: PureSpeed C18 Desalting tips loaded on Rainin's E4 XLS+ 8-channel electronic pipette

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