Evaluation of Different Empore™ StageTips for Proteomics Applications

Application Note

Proteomics

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Abstract

This application note describes the evaluation of three types of StageTips packed by CDS Analytical Empore™ membrane in the context of peptide desalting for proteomics research and demonstrates that comparable performance could be obtained from membranes with different chemical properties.

Introduction

Empore™ Solid Phase Extraction (SPE) micropipette StageTips are now available pre-packed with the Empore™ membrane. For almost 30 years, the Empore™ membrane has been the SPE product of choice to pack into micropipette tips for rapid, small-volume desalting and fractionation of peptides and proteins.[1,2] Pre-packed StageTips now offer researchers a much-needed alternative to arduous and monotonous manual packing procedures. Here the experiments by using different Empore™ pre-packed StageTips and a competitor's C18-based StageTips have been performed to evaluate their performance for proteomic sample preparation.

Experiment Setup

1. Materials and Equipment

(1) Empore StageTips: C18, 22% carbon loading, almost complete end-capping; Lot #:20001C. CDS Analytical Model # 6091, Fisher part #: 13-110-056; VWR part #:76449-262; SDB-XC, CDS Analytical Model #: 6093, Fisher part #: 13-110-060; VWR part #:76449-266; C18, 18% carbon loading, middle end-capping, R&D product. Lot #:20001CB. A competitor's C18 StageTips- C18 media, ~17% carbon loading, high end-capping; packed in house, 2-layer.
(2) Pipette tip adaptors (The Nest Group, Inc., Southborough, MA).
(3) Microtubes and Pipette tips: Axygen Maxymum Recovery tubes (1.5-mL and 2.0-mL); Woodpecker low-binding tips (20-μL, 200-μL, and 1.0-mL).
(4) Solutions:
Activation buffer: 100% methanol.
Wash and equilibration buffer: 0.5% acetic acid (HAc) in water.
Elution buffer I: 0.5% acetic acid, 60% acetonitrile (ACN) and 40% water.
Elution buffer II: 0.5% acetic acid, 80% ACN and 20% water.

Table 1. Different types of StageTips evaluated as part of this application note.

Experiment #	Type	Replicate
1	Competitor C18 StageTips	Competitor C18-1
2		Competitor C18-2
3		Competitor C18-3
4	Empore™ C18 Type C StageTips (CDS 6091)	C18-20001C-1
5		C18-20001C-2
6		C18-20001C-3
7	Empore™ C18 Type CB StageTips (R&D product)	C18-20001CB-1
8		C18-20001CB-2
9		C18-20001CB-3
10	Empore™ SDB-XC StageTips (CDS 6093)	SDB-XC-1
11		SDB-XC-2
12		SDB-XC-3

2. Sample Preparation

HeLa protein digest was obtained from Pierce. The lyophilized peptide mixture was resuspended into 0.1% formic acid to make final concentration of 100 ng/μL. Around 1 μg peptides were used for each experiment.

(1) StageTip pre-treatment: put StageTip and adaptor to 2-mL microtube.
(2) Activation I: Load 200 μL Methanol, spin @ 4000 rpm for 1~2 min.
(3) Activation II: Load 200 μL 80% ACN/0.5% HAc, spin 4000 rpm for 1~2 min.
(4) Discard all the liquid in the collection tube.
(5) Equilibration: Load 200 μL H_2O/0.5% HAc, spin 4000 rpm for 1~2 min.
(6) Discard all the liquid in the collection tube.
(7) Loading: Load 10 μL sample into tip (about 1 μg of peptides), spin 4000 rpm for 30 s; collect the flow through and reload onto tip, spin again; repeat this step three times.
(9) Washing: Load 200 μL H_2O/0.5% HAc, spin 4000 rpm for 2~3min; this step may be repeated 2~3 times. Also, depending on the salt amount, the spin time may vary (2~4 min).
(10) Transfer tips to new collection tubes.
(11) Elution I: load 200 μL 60% ACN/0.5% HAc, spin 4000 rpm for ~2 min.
(12) Elution II: load 200 μL 80% ACN/0.5% HAc, spin 4000 rpm for ~2 min; repeat this time one more time.
(12) Dry samples in a SpeedVac.

3. LC-MS conditions:

Analysis of peptide mixtures was performed on an Ultimate 3000 nano-LC and Orbitrap Eclipse mass spectrometer system coupled to a FLEX nano-electrospray ion source (all components were from Thermo Scientific). FAIMS Pro interface was enabled with a 165-min gradient, three CV experiment (-40, -60 and -80) was employed. Protein identification and quantification were performed using MaxQuant (version 1.6.3.4) and Perseus (version 1.6.2.3) software.

Results and Discussion

In this work, the Empore™ SPE membrane was utilized for peptide desalting as part of a typical shotgun proteomics pipeline. In total, four types of SPE membranes were examined, including the Empore™ C18 with near complete end-capping (Type C), C18 with moderate end-capping (Type CB), and SDB-XC. The Empore™ StageTips were then also compared to a competitor's product.

Example LC-MS profiles are shown in Figure 1. The LC-MS profiles indicate a highly reproducible chromatographic

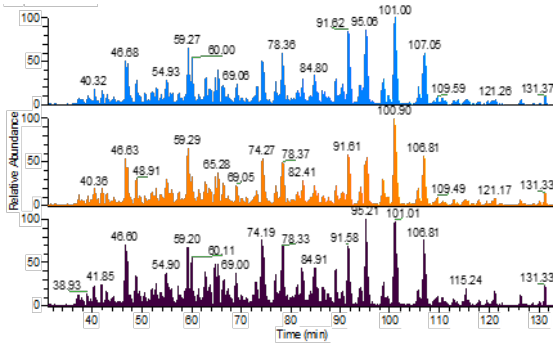

Figure 1. Representative base peak profiles of three replicate desalting experiments using Empore C18 Type CB StageTips, Lot # 20001CB.

separation, and was further verified by quantitative proteomics, as summarized in Figures 2-5. The evaluation for each type of StageTip was performed in triplicate. Figure 2 shows violin plots of proteins quantified by the MaxLFQ approach. The highly similar intensity distributions suggest excellent quantitative performance for all four types of StageTips.

Quantitative evaluation was further performed using the correlation plots in Figure 3. In each plot, two replicate experiments from StageTips of the same type are compared in the context of protein intensity to evaluate the tip-to-tip reproducibility. Quantified proteins showed high correlation (Pearson $r \geq 0.98$, $R^2 \geq 0.97$) between replicate tips and different lots, again suggesting excellent quantitative performance for all four types of StageTips. To evaluate the properties of identified proteins, isoelectric point (pI), and molecular weight (MW) were plotted among the four types of StageTips (Figure 4), which implicate that they did not show bias toward any particular MW size or specific pI ranges, indicating their potential for global proteome-wide quantification.

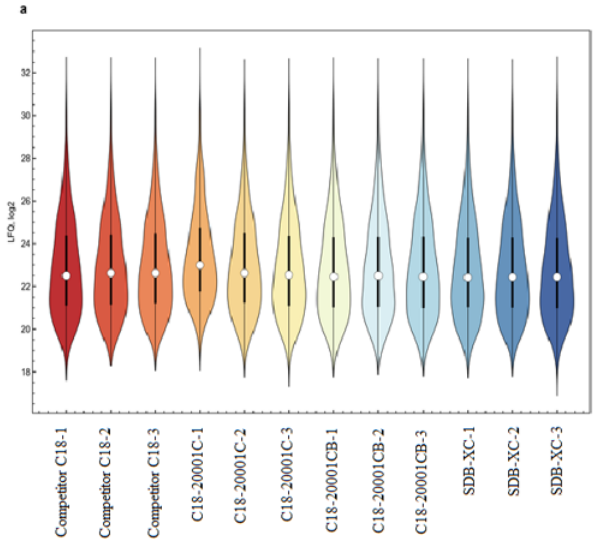

Figure 2. Violin plot of proteins quantified by MaxLFQ approach.

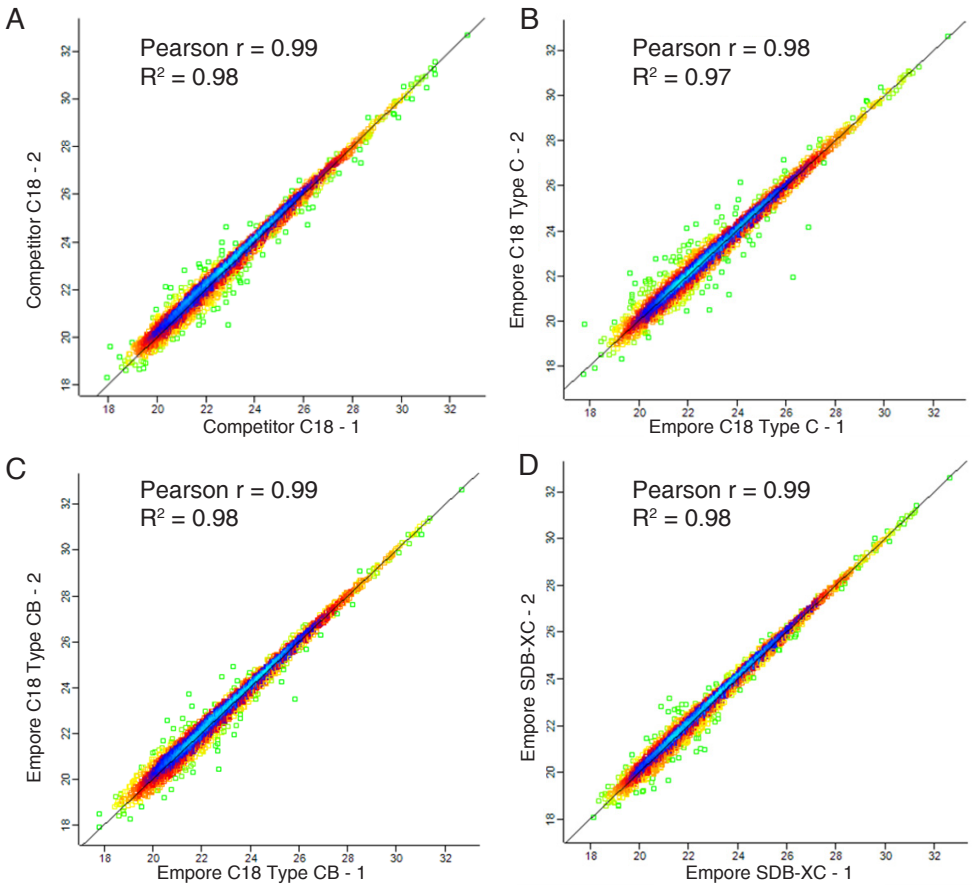

Figure 3. Density plots of protein intensities obtained by StageTips of (A) Competitor C18, (B) C18 Type C, (C) C18 Type CB, and (D) SDB-XC.

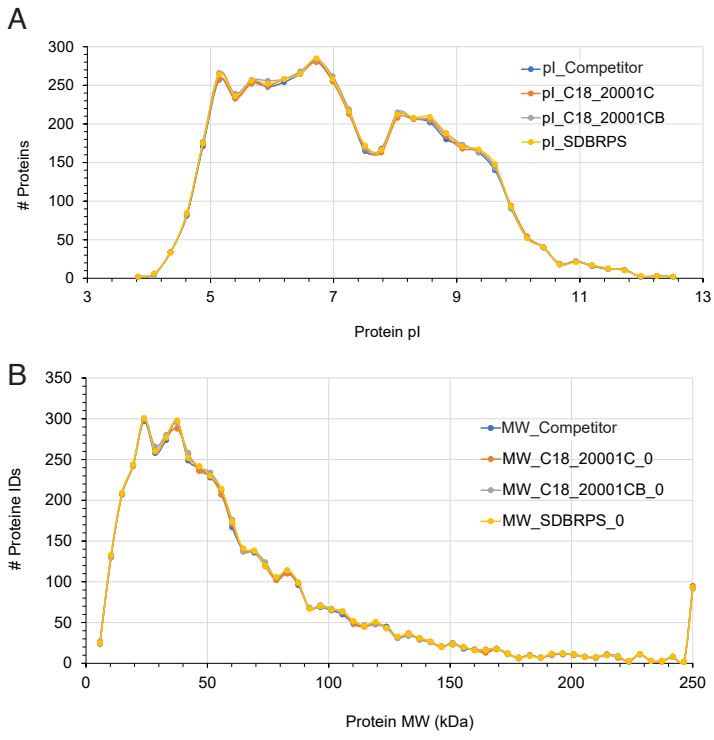

Figure 4. Comparison of the (A) pI and (B) MW of the proteins obtained by the four types of StageTips.

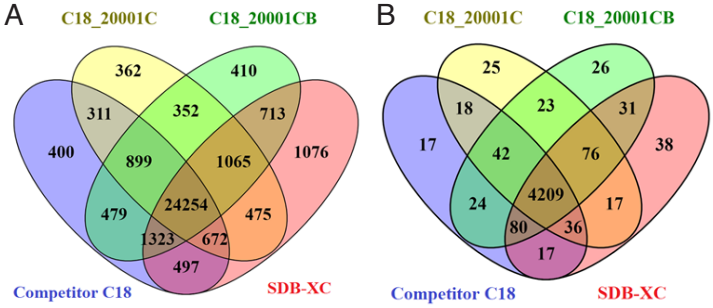

Figure 5. (A) Peptides and (B) proteins identified by all 4 types of StageTips: >92% proteins are identical.

Venn diagrams in Figure 5 show the overlaps of peptide (A) and protein (B) identifications of the StageTips. Over 92%, and nearly 81% of all protein and peptide hits, respectively, were commonly identified by each one of the four types of StageTips. Despite C18 Type CB having less end-capping and promoting more polar interactions between sorbent and peptide / protein, Type CB identified only 1% more peptide and proteins than C18 Type C. The SDB-XC StageTips identified the same number of peptides and proteins as the C18 Type CB. The data highlight again the consistent performance of Empore™ StageTips.

Conclusions

The data shown in this application note demonstrates that Empore™ StageTips are able to process peptide samples with excellent quantitative and qualitative performance and are comparable with competitor's product in the market. We envision that the Empore™ pre-packed StageTips will greatly facilitate proteome analysis in the context of clinical biomarker discovery, drug development, and mechanistic understanding of cell signaling.

References

1. Juri Rappsilber, Yasushi Ishihama, and Matthias Mann, Anal. Chem. 2003, 75, 663-670
2. Juri Rappsilber, Matthias Mann, and Yasushi Ishihama, Nature Protocol, 2007, 2, 1896-1906