CDS Empore™

Applying Empore™ E3technology™ to Proteomic Sample Preparation

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E3tips,10 µL

E3tips, 200 µL

E3filter,0.5 mL

E3plate,1.2 mL

Application Note

Life Science

Abstract

This note describes several applications of E3technology™ for proteomic sample preparation, which include two major components, protein cleanup and digestion. The note evaluated different formats of E3technology™, including E3tip, E3filter, and E3plate, and tested a variety of sample types, such as E. coli, yeast, mammalian cells, mouse tissues, and human body fluid. The technology has shown great advantages over existing methods in the context of proteome-wide identification and quantitation.

Experiment Setup

1. Materials and Equipment

(1) Empore™ E3tips, 10 µL : CDS Analytical, # 70-2019-3002-3
Empore™ E3tips, 200 µL : CDS Analytical, # 70-2019-3001-1
Empore™ E3filter, 0.5 mL: CDS Analytical, # 70-2019-3101-0
Empore™ E3plate, 1.2 mL: CDS Analytical, # 70-2019-3201-9

(2) Microtubes and Pipette tips: Low binding or maximum recovery microcentrifuge tubes (1.5 mL and 2.0 mL), Low binding tips (20 µL, 200 µL, and 1 mL).

(3) Solutions:

- Cell lysis:
  SDS buffer: 4% SDS, 100 mM Tris-HCl, pH 8.0
  Trifluoroacetic acid (TFA)
  Tris(2-carboxyethyl) phosphine hydrochloride (TCEP): 1.0 M stock
  Chloroacetamide (CAA): 1.0 M stock
- Protein digestion:
  Rinse/precipitation/wash (RPW) buffer: 80% acetonitrile (ACN)
  Digestion buffer: 50 mM Triethylammonium bicarbonate (TEAB, made from 1.0 M stock)
- Peptide elution:
  Elution I: 0.2% formic acid in water
  Elution II: 0.2% formic acid and 50% ACN in water

2. General Experimental Procedure:

(1) Cell lysate: collect cell pellets (E. coli, HEK293, yeast, etc.), and rinse two times with PBS. Collect mouse kidney tissue and human saliva following approved procedures. Generate lysate by adding 100-200 µL of SDS buffer, and boil with 10 mM TCEP/40 mM CAA (final concentration) at 95°C for 10 min. For TFA lysate, add 50-100 µL of pure TFA to cover cell pellets, and incubate at room temperature for 3-5 min.
(2) Protein precipitation and cleanup: add 4x volume of RPW buffer, transfer protein precipitates to E3tip, E3filter, E3, or E3plate. Spin to discard flow through. Wash 2-3 times with RPW buffer, and discard flow through.
(3) Protein digestion and peptide elution: add 100-200 µL of digestion buffer and

an optimized ratio of proteolytic enzyme. Then incubate filters at 37°C for 4-15 hours with gentle shaking.To elute peptides, add sequentially Elution I and II, spin, and collect elution. Dry peptides in SpeedVac.

(4) Peptide desalting: Please refer to NOTE # 278 (Evaluation of Empore™ C18 Spin Columns for Peptide Desalting and Proteomic Analysis), NOTE #259 (Evaluation of Different Empore™ Stag-eTips for Proteomics), and NOTE #272 (Evaluation of the Sample Loading Amount for Four Types of StageTips on Total Number of Protein Identifications in Proteomics) Applications for details.

(5) Proteome identification and quantification: LC-MS/MS was performed using an Ultimate 3000 RSLCnano system coupled with an Eclipse Orbitrap mass spectrometer. A linear gradient of 110 min from 1% to 35% buffer B (0.1% formic acid in acetonitrile) was employed, and spectra were acquired in data-dependent mode. Proteome Discoverer (version 2.5) and MaxQuant were used for protein database search with most of the default settings.

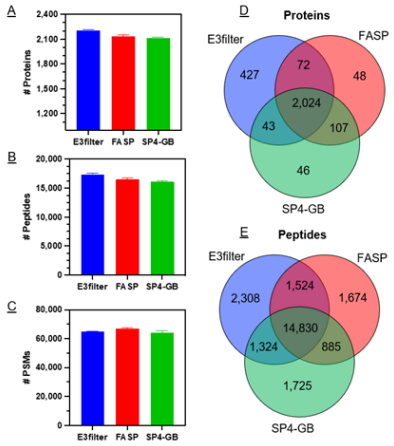

Figure 1. Qualitative assessment of E3technology™ (E3filter) for E. coli proteome analysis. (A-C) Comparison of the number of proteins, peptides, and PSMs between the E3filter, FASP, and SP4-GB approaches. Error bars represent three replicates. (D-E) Overlapping analyses of proteins and peptides derived from the three methods.

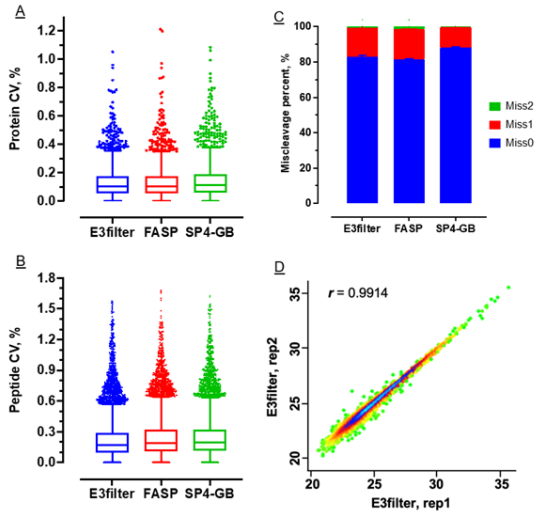

Figure 2. Quantitative assessment of E3technology™ (E3filter) for E. coli proteome analysis. (A) Coefficient of variation of quantified proteins by the three methods. (B) Coefficient of variation of quantified peptides. (C) Percentages of missed cleavages. (D) Pearson correlation of replicate experiments of E3technology™.

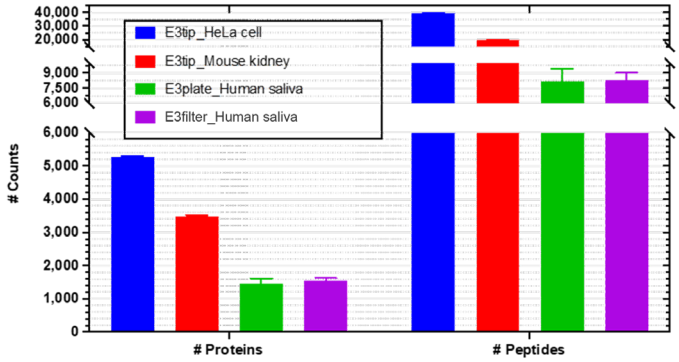

Figure 3. Applying E3technology™ to various sample types. Mammalian cells (HeLa), tissue (mouse kidney), and body fluid (human saliva) were tested using either E3tip, E3filter, or E3plate. Histograms showed the identification rates of unique proteins and peptide groups. Error bars represent biological replicates.

Results and Discussion

This application note benchmarks the performance of E3technology™ using different formats with a variety of samples of different complexity, volume, and quantity. We compared them side-by-side with several established methods, including FASP, SP4, SPEED, and S-tip. Our data suggests that E3technology™ provided equivalent or better performance in terms of proteome identification and quantitation. The reproducibility of the technology is generally high (Pearson > 0.95). E3technology™ is rapid, reliable, reproducible, easy-to-handle, and stress-free. It can be easily scaled and automated using our plate format. More importantly, E3technology™ significantly lowers technical and economic barriers to proteomics experiments. This note offers a powerful and versatile platform for proteome science.

Empore™ E3technology™ products.

Product Name	Part Number
E3tips, 10 µL	70-2019-3002-3
E3tips, 200 µL	70-2019-3001-1
E3filter, 0.5 mL	70-2019-3101-0
E3plate, 1.2 mL	70-2019-3201-9

Related Empore™ products.

Product Name	Part Number
C18 StageTips, 10 µL	70-2019-1019-0
C18 StageTips, 200 µL	70-2019-1001-3
C18 Spin Columns, 0.5 mL	70-2019-2001-0