Empore™ E3technology Proteomic Sample Preparation

January 2024

Protein digestion of **cell lysate** with **E3filter**

Product Description

E3technology™, introduced as the latest addition to the Empore™ E-series is an efficient, effective, and economical approach for proteomics sample preparation. Its accessibility makes it suitable for users of any expertise level. It swiftly conducts protein cleanup, preparing them for digestion in a matter of minutes, while eliminating detergents and interferences. Significantly, it provides a cost-effective solution, and following the cleanup, it seamlessly transitions to the digestion process within the same column.

Contents

Item	Part No.	Quantity
Empore™ E3tip 200 µl	70-2019-3001-1EA	25/PK
	70-2019-3001-1	96/Case
Empore™ E3tip 10 µl	70-2019-3002-3EA	25/PK
	70-2019-3002-3	96/Case
Empore™ E3filter 0.5 mL	70-2019-3101-0EA	25 spin columns and 25 2mL collection tubes in a pack
	70-2019-3101-0	100 spin columns and 100 2mL collection tubes in a case
Empore™ E3cartridge 3 mL	70-2019-3103-4EA	50/PK
	70-2019-3103-4	150/Case
Empore™ E3plate 1.2 mL	70-2019-3201-9EA	1/PK
	70-2019-3201-9	12/Case

Preparation

- Collect samples such as cell pellets, tissues, body fluids, etc.
- Lysis samples with buffers of your own choice.
- Estimate protein concentration and an aliquot certain amount for proteomics.
- Loading capacity: E3tip, ≤20 µg; E3filter, 10-100 µg; E3cartridge, 50-500 µg; E3plate, 30-200 µg.

Operation Steps

(general procedures for all four filter types; please adjust volume and centrifugation speed according to your filter type)

1. **Protein precipitation**
   Depending on sample volume, add 4x volume of 80% acetonitrile to induce protein precipitation.
   Note: organic solvents such as cold acetone or 90% methanol can also be used to induce protein precipitation.

2. **Sample loading**
   Transfer protein precipitate to E3filters, centrifuge at 2,000-4,000 rpm for 1-2 min; discard flow through.

3. **Wash**
   Add 200 µl organic solvents, and centrifuge at 2,000-4,000 rpm for 1-2 min. Discard flow through and repeat this step 2-3 times.

4. **Reduction and alkylation**
   Add 100-500 µl 50 mM triethylammonium bicarbonate (TEAB), 10 mM Tris(2-carboxyethyl) phosphine (TCEP), and 40mM chloroacetamide (CAA), incubate at 45°C for 5 min with gentle shaking.

5. **Wash**
   Centrifuge E3filters at 2,000-4,000 rpm for 1-2 min. Discard flow through. Add 100-500 µl 50 mM TEAB), and centrifuge at 2,000-4,000 rpm for 1-2 min. Discard flow through. The wash step may be repeated 2-3 times in total.

6. **Digestion**
   Transfer E3filters to clean collection tubes, and add 50-200 µl 50 mM TEAB, desired enzyme (Trypsin or Trypsin/Lys-C mix) at a 1:50 ratio. Incubate E3filters at 37°C for 16-18 hours with gentle shaking.

7. **Elution**
   Centrifuge E3filters at 2,000-4,000 rpm for 1-2 min, transfer elutes to new collection tubes. Perform two additional elution steps with 50mM TEAB, and 50% acetonitrile/0.1% formic acid in water, respectively. Pool the elution, dry, and proceed to desalting, or store at -80°C until further use.

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