Empore™ E4technology Proteomic Sample Preparation

January 2024

Protein digestion of **Intact Cells** with **E4filter**

Product Description

E4technology™ is designed to process intact cell (or tissue) samples directly for proteomics analysis. It avoids the lysis step and digests proteins directly in the fixed cells followed by peptide desalting (or fractionation) and elution. This all-in-one technology could drastically improve the sensitivity for low-cell or single-cell proteomics.

Contents

Item	Part No.	Quantity
Empore™ E4tip 200 µl	70-2019-3003-5EA	25/PK
	70-2019-3003-5	96/Case
Empore™ E4tip 10 µl	70-2019-3004-7EA	25/PK
	70-2019-3004-7	96/Case
Empore™ E4filter 0.5 mL	70-2019-3102-2EA	25 spin columns and 25 2mL collection tubes in a pack
	70-2019-3102-2	100 spin columns and 100 2mL collection tubes in a case
Empore™ StageTips Ring Adaptors	70-2019-1021-3	25/PK

Preparation

- Collect samples such as cell pellets and tissues. Rinse with cold PBS to remove excess culturing media or blood.
- Count cells and aliquot a certain amount for proteomics.
- Estimated capacity: E4tip, ≤ 50,000 cells; E4filter, 10,000-100,000 cells.

Operation Steps

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

1. **Cell treatment**
   Depending on the sample volume, add 2x pure methanol (100%) to the sample tube, and mix cells by gentle pipetting or vertexing.
   Note: when handling a small number of cells (e.g., <10,000 cells) using E4tip, cell samples can be loaded directly to the tip before adding methanol.

2. **Sample loading**
   Transfer cells to E4filters, centrifuge at 4,000 rpm for 1-2 min, and discard flow through.

3. **Cell fixing**
   Add 200-500 µl methanol (90%) and incubate in ice or at 4°C for 0.5-2.0 hours.

4. **Wash**
   Centrifuge at 4,000 rpm for 1-2 min. Discard flow through. Add 200-500 µl methanol (90%), centrifuge again, and discard flow through.

5. **Digestion**
   Transfer E4filters 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 at 37°C for 16-18 hours with gentle shaking.

6. **Reduction and alkylation**
   Add 10 mM Tris (2-carboxyethyl) phosphine (TCEP) and 40 mM chloroacetamide (CAA) and incubate at 70°C for 30 min with gentle shaking.

7. **Wash**
   Add acetic acid to the final concentration of 1%, centrifuge at 4,000 rpm for 1-2 min, and discard flow through. Add 200-500 µl 0.5% acetic acid in water, and centrifuge at 4,000 rpm for 1-2 min. Discard flow through.

8. **Elution**
   Transfer E4filters to clean collection tubes. Do two sequential elution by adding 200-500 µl 60% acetonitrile/0.5% acetic acid in water (elution I), and 80% acetonitrile/0.5% acetic acid in water (elution II), centrifuge at 2,000-4,000 rpm for 1-2 min, pool the elution, dry, store at -80°C until LCMS analysis.

CDS Analytical Headquarters:
465 Limestone Road P.O. Box 277 Oxford,
PA 19363-0277
Tel: 800.541.6593 610.932.3636
www.cdsanalytical.com