Empore™ E3technology Proteomic Sample Preparation

July 2024

Protein digestion of **cell lysate** with **E3plate™**

Product Description

E3technology, introduced as the latest addition to the Empore™ E-series is an efficient, effective, and economical approach for proteomics sample preparation. The multi-well format of the technology, E3plate™, provides a high throughput solution for large-scale proteome analysis. In particular, combined with Empore™ desalting plate, it significantly streamlines sample preparation, from crude protein lysate to clean LCMS-ready peptides.

Contents

Item	Part No.	Quantity
Empore™ E3plate 1.2 mL	70-2019-3201-9EA	1/PK
	70-2019-3201-9	12/CS
Desalting Plate	**Part No.**	**Quantity**
Empore™ C18-SD plate, 1.2 mL	70-2007-3982-2EA	1/PK
	70-2007-3982-2	12/CS
Empore™ SDB-XC plate, 1.2 mL	98-0405-0081-4EA	1/PK
	98-0405-0081-4	12/CS
Empore™ SDB-RPS plate, 1.2 mL	98-0405-0080-6EA	1/PK
	98-0405-0080-6	12/CS

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.
- Recommend loading capacity: 30-500 μg.

Operation Steps

1. Sample loading and protein precipitation
   Transfer protein lysate directly to the plate, if the sample volume is 200μl or less. Then, add 4x volume of 80% acetonitrile to induce protein precipitation.
   *Note: if the sample volume is >200μl, please do precipitation in separate tubes, and then transfer protein precipitates to E3plate.

2. **Wash**
   Centrifuge at 100 x g for 1-2 min; discard flow through. Depending on the size of the collection plate, add 200-500μl of 80% acetonitrile, and spin again.
   *Note: speed may go up to 1,500 x g if protein input is large.

3. Reduction and alkylation
   Add 200 μl of 50 mM triethylammonium bicarbonate (TEAB) with a final concentration of 10 mM Tris(2-carboxyethyl) phosphine (TCEP), and 40mM chloroacetamide (CAA), incubate at 45°C for 10 min with gentle shaking.
   *Note: this step may be skipped here if performed upfront during cell lysis.

4. Wash
   Centrifuge E3filters at 200 x g for 1-2 min. Discard flow through. Add 200 μl 50mM TEAM and wash again. The wash step may be repeated 2-3 times in total.

5. Digestion
   Add 200μl 50 mM TEAB, and desired enzyme (Trypsin or Trypsin/Lys-C mix) at a 1:50 ratio. Incubate at 37°C for 16-18 hours with gentle shaking.
   *Note: shaking is optional.

6. Elution, and desalting
   Here, instead of doing "elute-dry-resuspend" and then a separate desalting step, we integrate the peptide elution step from the E3plate, and the desalting step with a C18-based plate, into a single step to streamline the process and minimize sample loss.

   1). Acidification: after digestion, add 2% formic acid (final concentration) to the E3plate, and leave it on the bench for a few minutes.
   2). C18 plate pretreatment: In the meantime, activate a C18 plate by adding 200μl of methanol and spinning at 800 x g for 1-2min. Then, equilibrate the plate with 200μl of 0.5% acetic acid in water, spin, and discard flow through.
   3). Load: stack E3plate onto C18 plate and a collection plate, spin at low speed (200~400 x g) for 5-10min, then discard flow through.
   4). Wash: Add 200μl of 0.5% acetic acid in water, spin, and discard flow through.
   5). Elute: transfer the E3 and C18 to a new collection plate, elute peptides sequentially with 200μl of 60% and 80% acetonitrile/0.5% acetic acid in water, spin and collect flow through, dry, and proceed to LCMS, or store at -80°C until further use.

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