Instructions to Proteonano™ PAC Proteome Extraction Kit

- Operational Manual

Cat: SP3K001 Specifications: 96 tests/kit

Cat: SP3K003 Specifications: 8 tests/kit

1 Introduction

A pivotal phase in proteomics research involves the effective extraction and handling of protein samples to guarantee maximum sensitivity in subsequent detection. To accomplish this, a sample handling technique is necessary that provides unbiased handling of proteins, adaptability in the use of reagents, and near-zero loss during processing. In response to these requirements, the single-pot, solid-phase-enhanced sample preparation (SP3) method is an innovative approach based on paramagnetic beads that enables swift, reliable, and efficient processing of proteins for proteomic studies. The SP3 technique employs a hydrophilic interaction process to replace or eliminate substances typically used to aid in cell or tissue lysis, protein solubilization, and enzymatic breakdown (such as detergents, chaotropes, salts, buffers, acids, and solvents) prior to downstream proteomic analysis.

2 Kit Components

All products and services described in this document are for research use only and are not intended for diagnostic procedures.

Reagents	Cat. Specifications (96 tests)	Cat. Specifications (8 tests)	Storage Temperature	Notes
Hydrophilic Mag-beads	SP3001 0.5 mL/vial	SP3001 0.05 mL/vial	2-8 °C	Protein extraction
Hydrophobic Mag-beads	SP3002 0.5 mL/vial	SP3002 0.05 mL/vial	2-8 °C	Protein extraction
One-step Digestion Buffer	BD001 5 mL	BD001 1 mL	2-8 °C	Protein denaturation, reduction and digestion

Enzyme	ENM-A-120 120 ug*2 (Powder)	ENM-A-20 20 ug (Powder)	2-8 °C	Powder of Trypsin/Lys-C Mix
Ending Buffer	BT001 2 mL	BT001 0.2 mL	2-8 °C	Stop digestion
Activating Buffer	BA001 20 mL	BA001 4 mL	2-8 °C	Activate C18 pipette tip
Wash Buffer	BW001 30 mL * 2	BW001 10 mL	2-8 °C	Clean C18 membrane
Elution Buffer	BE001 15 mL	BE001 4 mL	2-8 °C	Elute peptides from C18 membrane
Resuspend Buffer	BR001 2 mL	BR001 1 mL	2-8 °C	Peptide powder reconstitution solution
Desalting Tips	MC18TB 96 pcs	MC18TB 8 pcs	RT	C18 desalting tips

3 Instrument and consumables preparation

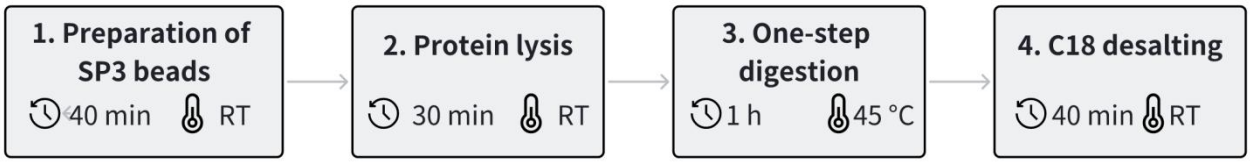
Equipment and Consumables	Diagram	Example	Notes
Ultrasonic Cleaning Bath		ThermoFisher 15-337-411 or equivalent	Used for dispersion of mag-beads
Vortex		Kylin-Bell VORTEX-5 or equivalent	Used for dispersion of mag-beads
Magnetic rack		ThermoFisher 12321D or equivalent	Used for collection of mag-beads

Heater shaker		ThermoFisher 88880028 or equivalent	Provides constant temperature and shaking during incubation
Vacuum freezer		Telstra Lyoquest 85 or equivalent	Used for concentrating peptides after desalting
Low protein binding tips & tubes		ThermoFisher 88379 or equivalent	Minimizes protein loss during operation

4 Reagent required but not provided

Reagent		Note
Deionized water	/	Used for magnetic bead washing and reagent preparation in the reagent preparation step
Absolute ethanol		Sigma 1000302500 GC ⩾99.5%
80% ethanol		Prepare with absolute ethanol
Desalting tips adapter		Nanomics Cat.: TRAA

5 Experimental Procedures



5.1 Reagent Preparation

1. Mix 5 µL * (number of samples + 1) of **Hydrophilic Mag-beads** and **Hydrophobic Mag-beads** respectively (each test requires 5 µL of each of the two types of magnetic beads, totalling 10 µL).
2. Take 50 µL * (number of samples + 1) of deionized water to wash the **mixed magnetic beads**, shake for 10 seconds, and place on a magnetic stand for 2 minutes until the liquid is clear, then discard the supernatant.
3. Repeat the washing step twice.
4. Take 50 µL * (number of samples + 1) of deionized water to resuspend the **mixed mag beads**.

! Note: Magnetic beads need to be cleaned immediately before use. Use the cleaned beads in a short time after discarding the supernatant to avoid drying of the magnetic beads.

5.2 Protein Extraction and Digestion

1. Take a clean 2 mL EP tube and add the cell lysate containing 50 µg of protein. please use the lysis buffer to make up the volume to 100 µL.

! For low protein concentrations with sample volumes over 100 µL, take the sample according to the actual needs.

2. Add 50 µL **mixed mag beads** to the protein samples. Vortex to mix, then add an equal volume of Acetonitrile(150 µL or the actual sample volume)), and incubate at room temperature 25 °C, 1500 rpm, for 10 min.
3. After incubation, magnetically separate and discard the supernatant. Wash three times with 180 µL of 80% ethanol.
4. Add 5 mL (for 96× format) or 0.5 mL (for 8× format) of deionized water to the O**ne-step Digestion Buffer** tube and mix thoroughly.
5. Transfer 4.8 mL (when using 96x kit) or 0.4 mL (when using 8x kit) of **One-step Digestion Buffer** into the bottle of **Enzyme** and vortex to fully dissolve the **Enzyme** . After the Absolute ethanol has dried, add 50 µL dissolved **Enzyme** . Incubate and shake at 1500 rpm and 45 °C for 1 hours.

6. After incubation, briefly centrifuge to collect liquid at the bottom of the tube, then add 20 µL of **Ending Buffer** to terminate the digestion.
7. Collect beads on the magnetic rack for 2 min, and take the supernatant for desalting, after the liquid is clear.

5.3 Desalting and Lyophilization

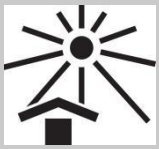
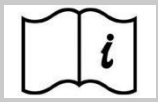
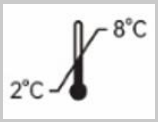
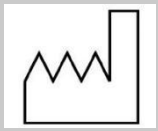
1. Assemble desalting tip on microfuge tube.
2. Add 200 µL **Activating Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature, then discard the **Activating Buffer**.
3. Add 200 µL **Wash Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature, then discard the **Wash Buffer**.
4. Add 70 µL of the digested protein sample prepared in 7.2 Denaturation, Reduction, Alkylation, and Digestion step 3 to the desalting tip prepared in the last step. Centrifuge at 1200 × g for 3 minutes at room temperature and discard the flow through.
5. Add 100 µL **Wash Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature, discard the flow through. Repeat the step twice, for a total of three washes.
6. Add 50 µL **Elution Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature, collect the eluent.
7. Add additional 50 µL **Elution Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature. collect the eluent.
8. Add additional 50 µL **Elution Buffer** to the desalting tip. Centrifuge at 1200 × g for 3 minutes at room temperature, collect and combine the total 150 µL of eluent from this step and the last two elution steps.
9. Completely dry the eluent by using a Vacuum Freezer or equivalent equipment.
10. Add 20 µL **Resuspend Buffer** to dissolve lyophilized peptide powder.
11. Store at -80 °C until peptide concentration measurement and LC-MS analysis

5.4 Peptide Concentration Measurement

1. Resuspend lyophilized peptide with 20 µL **Resuspend Buffer**.

2. Take 1 µL of resuspended sample and measure the absorbance at 205 nm (A205) using a microvolume spectrophotometer (ThermoFisher NanoDrop One or equivalent)
3. Calculate the peptide concentration (µg/µL) as A205 ÷ 31.

Label introduction for user:

Abbreviation	Explanation	Abbreviation	Explanation
	Catalogue number		Keep away from sunlight
	Batch code		Consult instructions for use
	Temperature limit: 2~8°C		Date of manufacture
	Use-by date		Manufacturer
	CE mark		
	Nanomics Biotechnology Co., Ltd. Address: 5th Floor, Building 6, 400 Fucheng Road, Xiasha Street, Qiantang District, Hangzhou City, Zhejiang Province, 310000 Tel: +86 0571-85063539 Email: support@nanomics.bio Web: www.nanomics.bio		
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