Instructions to Proteonano™ SP3 Proteome Extraction Kit

- Operational Manual

Cat: SP3K001 Specifications: 96 tests/kit

Cat: SP3K003 Specifications: 8 tests/kit

Nanomics

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

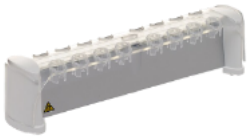
All products and services described in this document are for research use only and are not intended for diagnostic procedures.

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
Reducing Reagent	BRE001 1.9 mL	BRE001 0.2 mL	2-8 °C	Protein reduction
Alkylation Reagents	BAL001	BAL001	2-8 °C	Protein alkylation

	1.5mL	0.2 mL		
Rapid Trypsin	PRT-120 120 ug (Powder)	PRT-20 20 ug (Powder)	2-8 °C	Powder of Rapid Trypsin
Digestion Buffer	BD003 5 mL	BD003 0.5 mL	-20 °C	Protein digestion
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	超声分散富集试剂中的纳米磁珠
Vortex		Kylin-Bell VORTEX-5 or equivalent	

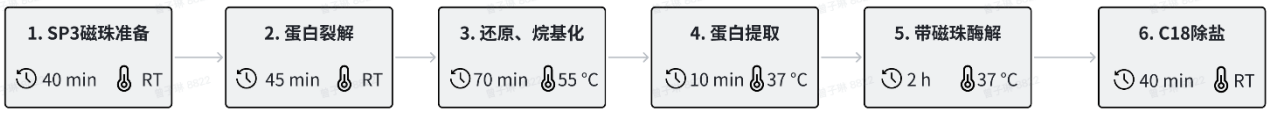
Magnetic rack		ThermoFisher 12321D or equivalent	用于分离富集试剂中的纳米磁珠
Heater shaker		ThermoFisher 88880028 or equivalent	提供孵育过程中的恒温和振荡环境
Vacuum freezer		Telstra Lyoquest 85 or equivalent	用于除盐后的肽段浓缩
Low protein binding tips & tubes		ThermoFisher 88379 or equivalent	减少操作过程中的蛋白质吸附

4 Reagent required but not provided

Reagent	Diagram	Note
Deionized water	/	Used for magnetic bead washing and reagent preparation in the reagent preparation step
Absolute ethanol		Sigma 1070172511 GC ⩾99.5%。
80% ethanol		Prepare with absolute ethanol
Desalting tips adapter A		珞米货号：TRAA

Desalting tips adapter B		与单根除盐枪头适配器二选一即可，珞米货号：THRAC

5 Experimental Procedures



5.1 SP3 Beads Preparation

1. Nanomics Protein extraction magnetic beads are stored at 50 mg/mL concentration and should be stored at 4°C when not in use.
2. Take the beads out of storage at 4°C and let them equilibrate to room temperature for 30 minutes. If the beads have settled during storage, they should be resuspended by inversion or gentle vortexing until no solid bead mass is visible at the bottom of the bottle. Preparing aliquots of stock beads avoids excess handling of the main bottles and minimizes the risk of contamination.
3. Prepare a fresh 1:1 bead mix before each experiment by combining equal volumes of stock SP3001 and SP3002 beads in a clean Sarstedt tube.
4. Briefly vortex the mixture and place the tube on the magnetic stand for two minutes to collect the beads. Aspirate and discard the storage buffer.Wash the beads with ultrapure water at a volume corresponding to 5–10× the initial volume of mixed beads.
5. Vortex the beads for 10 seconds and place on the magnetic stand for two minutes to collect the beads. Aspirate and discard the wash buffer.
6. Repeat the wash steps two times.
7. Resuspend the beads with ultrapure water at a final concentration of 10 µg/µL.

5.2 SDS Cell Lysis

1. SDS lysis buffer: Prepare fresh 50 mM HEPES pH 8.5/1% SDS from stock 1 M HEPES pH 8.5 and 10% SDS solutions. Add cOmplete Mini EDTA-free Protease Inhibitors (Roche) to 1× concentration.
2. Thaw cell pellets on ice.
3. Add four pellet volumes of SDS lysis buffer to the cell suspension. Homogenize using a disposable pestle (Bel-Art) with 20 strokes.
4. Add benzonase to a concentration of 500 units/mL and mix by inversion.

5. Incubate the lysate at 4°C for 30 minutes with end-over-end rotation.

- Check the viscosity of the samples at this point by pipetting. If you are unable to pipette, add more benzonase and extend the incubation.

6. Pellet cellular debris by centrifugation at 16000 rcf for 10 minutes at 4°C and transfer the supernatant to a clean tube.
7. Quantify the protein content by BCA protein assay.
8. Normalize all samples to the same volume and concentration with SDS lysis buffer.

- Aliquots of lysate can be frozen and stored at -80°C for later use.

5.3 Reduction and Alkylation

1. Add DTT (100 mM stock) to a 10 mM final concentration and briefly vortex.
2. Reduce and denature the samples at 56°C for 30 minutes in a water bath.
3. Cool the samples to room temperature and centrifuge for 2 seconds to collect liquid from the sides of the tubes/caps.
4. Add IAA (550 mM stock) to a 22.5 mM final concentration and briefly vortex.
5. Alkylate the samples by incubating in the dark for 30 minutes at room temperature.
6. Add fresh 100 mM DTT to a final concentration of 10 mM to quench residual IAA and incubate for 10 minutes at room temperature.

5.4 SP3 Protein Cleanup

1. Check the pH of the sample is in the range of 7 to 8.5 for optimal binding by measuring an aliquot on pH paper.
2. Add washed beads (prepared as above) to the samples in a ratio of 5–10 µg of beads to 1 µg of protein and briefly vortex.
3. Immediately add a volume of 100% ethanol to the samples to obtain a 50% final concentration to initiate protein binding to the beads.
4. Vortex the samples to mix but ensure that beads are not stuck on the sides of the tube.

- Note: The protein-bead mixture will be sticky at this stage. Avoid touching the beads to minimize sample loss.

5. Incubate the samples on a room temperature mixer platform for 10 minutes at 1000 rpm.
6. Remove the samples from the mixer, centrifuge them for two seconds, and place them on the magnetic stand for two minutes.
7. Transfer the supernatants to a clean Sarstedt tube.

8. Wash the beads by adding a volume of 80% ethanol corresponding to at least twice the initial sample volume and vortex for 30 seconds.
9. Centrifuge the samples for two seconds and place back on the magnetic stand for one minute.
10. Remove the washes and save in a separate Sarstedt tube. Repeat two times.
11. After the final wash, air dry the beads for 30 seconds to remove as much ethanol as possible.
12. Resuspend the beads in 100 mM ammonium bicarbonate buffer and vortex.

- If the beads remain aggregated after vortexing, then briefly sonicate.

13. Add Promega™ sequencing grade trypsin in a 1:20 enzyme-to-substrate ratio to each sample.
14. Ensure the pH for all samples is in the range of 7.5 to 8 and digest at 37°C overnight.

- Digesting on a mixing platform or end-over-end rotator to keep the beads in suspension is recommended.

5.5 SP3 Peptide Cleanup

- Note: Peptide binding to SP3 beads is promoted by the addition of acetonitrile to a final concentration ⩾ 95%. The aqueous sample volume prior to dilution must not exceed the available tube volume (e.g., 50 µL of peptides would require the addition of 1 mL of 100% MeCN to reach ~95% MeCN concentration). If necessary, sample volume may be reduced by vacuum concentration prior to addition of MeCN. Complete drying to avoid potential sample loss is recommended against.

1. Remove the samples from incubation at 37°C and centrifuge for one minute at 2000 rcf to pellet the beads.
2. Incubate the digested samples on the magnetic stand for two minutes and transfer the supernatant to clean Sarstedt tubes.

- Save an aliquot of the digested peptide supernatant for quality control analysis by fluorometric peptide assay and store at -80°C until needed.

3. Ensure the sample pH is still in the range of 7.5 to 8 after overnight digestion.
4. Add washed beads (prepared as above) to the samples in a ratio of 5–10 µg of beads to 1 µg of peptide and briefly vortex.
5. Immediately add a volume of 100% MeCN to reach ⩾ 95% final concentration to initiate peptide binding to the beads.
6. Immediately vortex to maintain beads in suspension.

- Concentrated peptide samples may cause the beads rapidly aggregate together or to the side of the tube after the addition of MeCN. If this occurs, briefly sonicate the tube and vortex until the beads are back in suspension.

7. Incubate all samples on a room temperature mixer for 10 minutes at 1000 rpm.
8. Remove the samples from the shaker and centrifuge for two seconds to collect any liquid or beads on the sides or cap.
9. Place the samples on the magnetic stand for two minutes.
10. Remove the supernatant and save in a clean Sarstedt tube.
11. Wash the beads with a volume of 100% MeCN corresponding to at least two times the initial sample volume and vortex for 30 seconds.
12. Centrifuge the samples for two seconds and place on the magnetic stand for one minute.
13. Aspirate the supernatant and save in a separate tube.
14. Air dry the beads for 30 seconds to ensure MeCN is removed before starting the elution.
15. Elute peptides with 2% DMSO in water in a volume equivalent to 5–10× the dried bead volume.

- Add the elution buffer to the dried beads; avoid touching the beads with the pipette tip to avoid sample loss.

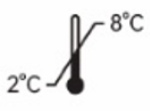
16. Sonicate the samples for one minute in a bath sonicator to disrupt any aggregated beads and to promote the release of bound peptides.
17. Incubate the samples on a room temperature mixer platform for five minutes at 1000 rpm.
18. Remove the samples from the mixer and centrifuge for two seconds.
19. Place them on the magnetic stand for two minutes.
20. Optional step to prevent bead carryover: aspirate the eluates as above and transfer to new tubes. Incubate on the magnetic stand for two minutes and transfer the supernatants to clean tubes.

- Save an aliquot of the eluate for quality control analysis by fluorometric peptide assay and store at -80°C until needed.

21. Samples are ready for immediate analysis by LC-MS/MS or storage at -80°C.

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		

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