Instructions to Proteonano™ Plasma Proteome Enrich Kit

Nanomics
The Next-Gen Proteomics Company

- Operational Manual

Cat: PN001-3 Specifications: 48tests/kit

1.Introduction

The Proteonano™ technology uses peptides and small molecules modified on the surface of magnetic nanoparticles (multi-valent, multi-affinitive nanoprobes, MMNPs) for plasma/serum protein capture. This allows selective capture of low abundant proteins, resulting in relative depletion of high abundant proteins. This improves LC-MS/MS detection depth, promoting the effectiveness of untargeted proteomic analysis.

2.Kit Components

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

Reagents	Cat.	Specifications	Storage Temperature	Notes
Enrichment Nanobeads	NP003-16	3 vials 16 tests/vial	2-8 °C	Low-abundance protein enrichment magnetic nanobeads
EN-Binding Buffer	BF001	2 bottles 30 mL/bottle	2-8 °C	Promote the binding of protein and magnetic beads
Digestion Buffer 1	BD001	1 mL	-20 °C	Protein denaturation and reduction
Digestion Buffer 2	BD002	1 mL	2-8 °C	Protein digestion
Rapid Trypsin	BD003	50 ug (Powder)	2-8 °C	Powder of 50 ug Rapid Trypsin
Ending Buffer	BT001	1 mL	2-8 °C	Stop tryptic digestion
Activating Buffer	BA001	10 mL	2-8 °C	Activate C18 pipette tip
Wash Buffer	BW001	30 mL	2-8 °C	Clean C18 membrane
Elution Buffer	BE001	10 mL	2-8 °C	Elute peptides from C18 membrane

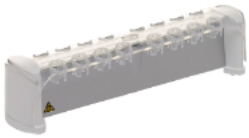
Resuspend Buffer	BR001	1 mL	2-8 °C	Peptide powder reconstitution solution
Desalting Tips	6091	48 pcs	RT	C18 desalting tips

3.Sample Preparation

1. Plasma/Serum Sample Pre-treatment: thaw samples on ice. Take 40 µL plasma/serum in a Low protein binding 2 mL tube*.

- *Low protein binding consumables should be used, including micropipette tips and microcentrifuge tubes.

4.Equipment and Consumables Required

Equipment and Consumables	Diagram	Example
Vortex		Kylin-Bell VORTEX-5 or equivalent
Magnetic tube rack		ThermoFisher 12321D or equivalent
Heater shaker		ThermoFisher 88880028 or equivalent
Refrigerated centrifuge		ThermoFisher ST16R or equivalent
Vacuum freezer		Telstra Lyoquest 85 or equivalent
Microvolume spectrophotometer		ThermoFisher NanoDrop One or equivalent

Low protein binding tips & tubes		ThermoFisher 88379 or equivalent

5.Important Preparation Steps

The performance of the Proteonano™ Plasma Proteome Enrich Kit is dependent on the maintenance and set up for LC-MS/MS, and proper execution of experimental steps outlined in section 6 below. Thus, it is critical to use Proteonano™'s quality control system (QCS) to test the performance of LC-MS/MS setup and familiarize with experimental procedures before performing proteomic experiments using experimental samples.

QCS system should also be used along with experimental samples to monitor kit and LC-MS/MS performance during sample processing and data acquisition.

Please contact Nanomics (support@nanomics.bio) for User guide of Proteonano™ quality control system (QCS).

6.Sample Quality and Storage Conditions

No significant hemolysis should occur for both plasma and serum samples. Although protein groups detected in samples with significant hemolysis, this may be caused by detection of blood cell proteins rather than plasma or serum proteins. This may negatively impact data analysis and interpretation.

Storage time and repeated sample freeze-thaw cycles both negatively impact protein groups detected by the assay. Whenever possible, samples with short storage times or samples with similar storage time should be used in the same experiment.

7.Experimental Procedures

7.1.Protein Enrichment

1. Kit Preparation: Equilibrate **Enrichment Nanobeads** at room temperature. Vortex to achieve a uniform suspension of **Enrichment Nanobeads**.
2. Add 40 µL **EN-Binding Buffer** to the plasma/serum, gently vortex to mix, and place the microcentrifuge in a microcentrifuge heater shaker, incubate at room temperature, shake at 1500 rpm for 5 minutes*.

- * Heater-shaker rotating speed may need to be adjusted due to variations in heater-shaker manufacturer. Please ensure **Enrichment Nanobeads** is sufficiently mixed without buffer splashing.

3. Add 20 µL prepared **Enrichment Nanobeads** to the plasma/serum in **EN-Binding Buffer**, gently vortex to mix, and place the microcentrifuge in a microcentrifuge heater shaker, incubate at room temperature, shake at 1500 rpm for 1 hour. *
4. After incubation, place the centrifuge tube on the magnetic rack for at least 3 minutes, * until **Enrichment Nanobeads** is completely collected by the magnet, and discard the supernatant by pipetting.

- * Due to variations in magnetic module manufacturer, preliminary test should be performed to determine the best duration for **Enrichment Nanobeads** immobilization steps in the protocol. To ensure complete **Enrichment Nanobeads** recovery, magnetic separation time can be extended to 10 minutes for the initial experiment.

5. Add 180 µL **EN-Binding Buffer** to the centrifuge tube, gently vortex to resuspend **Enrichment Nanobeads**, place the centrifuge tube on the magnetic rack for at least 3 minutes, until **Enrichment Nanobeads** is completely collected by the magnet, and discard the supernatant by pipetting. Repeat 2 times, remove supernatant completely after final wash*.

- *Protein enrichment is complete after this step. Enriched proteins absorbed by **Enrichment Nanobeads** do not need to be eluted. Proteins bound to **Enrichment Nanobeads** can be directly subjected to subsequent denaturation, reduction, alkylation, digestion, and desalting.

7.2.Denaturation, Reduction, Alkylation, and Digestion

1. Add 20 µL **Digestion Buffer 1** to the microcentrifuge tube containing **Enrichment Nanobeads** from the last step, incubate and shake at 1500 rpm at 55 °C on microcentrifuge tube heater shaker for 0.5 hours.
2. Brief centrifuge to collect reagent on the bottom of microcentrifuge tube. Allow the sample to return to room temperature.

3. Transfer 1 mL of **Digestion Buffer 2** into the bottle of **Enzyme** and vortex to fully dissolve the **Enzyme**. Add 20 µL dissolved **Enzyme**. Incubate and shake at 1500 rpm and 37 °C in the dark for 2 hours.
4. Add 20 µL **Ending Buffer** to stop tryptic digestion. Vortex to mix well and place the centrifuge tube on the magnetic rack for at least 3 minutes, until **Enrichment Nanobeads** is completely collected by the magnet. Transfer the supernatant (approximately 60 µL) by pipetting for subsequent of processing.

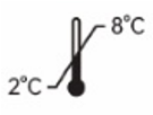
7.3.Desalting and Lyophilization

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 60 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

7.4.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℃		Date of manufacture
	Use-by date		Manufacturer
	CE mark		

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