Instructions to Proteonano™ EV Proteome Enrich Kit

- Operational Manual

Nanomics

Cat: EXSK001 Specifications: 48tests/kit

Cat: EXSK001-08 Specifications: 8tests/kit

1.Introduction

The Proteonano™ technology uses special designed probes modified on the surface of magnetic nanoparticles for EV capture. Beads can enrich EVs when incubated with samples by inserting the probe structure into the lipid bilayer of EVs. This improves LC-MS/MS detection depth, promoting the effectiveness of EV 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 (48 tests)	Cat. Specifications (8 tests)	Storage Temperature	Notes
EV Enrichment Nanobeads	EXS001-16 3 vials, 16 tests/vial	EXS001-08 1 vial, 8 tests/vial	2-8 °C	Low-abundance protein enrichment magnetic nanobeads
EN-Binding Buffer	BF001 30 mL	BF002 2 mL	2-8 °C	Promote the binding of protein and magnetic beads
EN-Wash Buffer	BW002 30 mL	BW002 15 mL	2-8 °C	Clean the non-specific binding proteins
One-step Digestion Buffer	BD001 1.3 mL*2	BD001 0.5 mL	2-8 °C	Protein denaturation、reduction and digestion
Enzyme	ENM-A-050 50 ug (Powder)	ENM-A-010 10 ug (Powder)	2-8 °C	Powder of Trypsin/Lys-C Mix
Ending Buffer	BT001 1 mL	BT001 0.4 mL	2-8 °C	Stop tryptic digestion

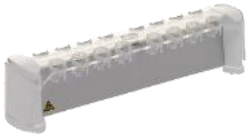
Activating Buffer	BA001 10 mL	BA001 4 mL	2-8 °C	Activate C18 pipette tip
Wash Buffer	BW001 30 mL	BW001 10 mL	2-8 °C	Clean C18 membrane
Elution Buffer	BE001 10 mL	BE001 4 mL	2-8 °C	Elute peptides from C18 membrane
Resuspend Buffer	BR001 1 mL	BR001 1 mL	2-8 °C	Peptide powder reconstitution solution
Desalting Tips	6091-48 48 pcs	6091-8 8 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*.

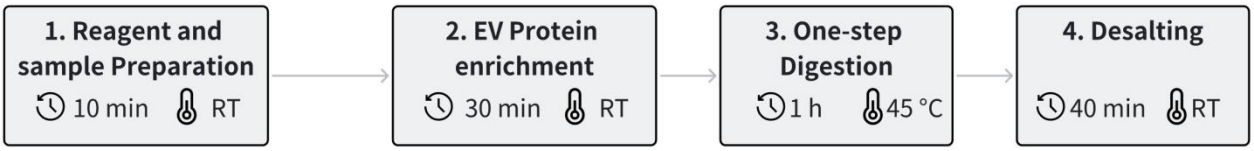
- *Low protein binding consumables should be used, including micropipette tips and microcentrifuge tubes.

4.Equipment and Consumables Required

Equipment and Consumables		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.Experimental Procedures



5.1.Protein Enrichment

1. Kit Preparation: Equilibrate **EV Enrichment Nanobeads** at room temperature. Vortex to achieve a uniform suspension of **EV 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 **EVEnrichment Nanobeads** is sufficiently mixed without buffer splashing.

3. Add 20 µL prepared **EV 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 0.5 hour.

4. After incubation, place the centrifuge tube on the magnetic rack for at least 3 minutes*, until **EV 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 **EV Enrichment Nanobeads** immobilization steps in the protocol. To ensure complete **EV Enrichment Nanobeads** recovery, magnetic separation time can be extended to 10 minutes for the initial experiment.

5. Add 180 µL **EN-Wash Buffer** to the centrifuge tube, gently vortex to resuspend **EV Enrichment Nanobeads**, place the centrifuge tube on the magnetic rack for at least 3 minutes, until **EV 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 **EV Enrichment Nanobeads** do not need to be eluted. Proteins bound to **EV Enrichment Nanobeads** can be directly subjected to subsequent denaturation, reduction, alkylation, digestion, and desalting.
- For the 48-test kit, there are two vials of One-step Digestion Buffer; add 1.3 mL of deionized water to each vial before use to ensure complete dissolution.

5.2.Denaturation, Reduction, Alkylation, and Digestion

1. Add 1.3 mL (for 48 tests) or 0.5 mL (for 8 tests) of deionized water to each vial of **One-step Digestion Buffer** and mix thoroughly.

- For the 48-test kit, there are two vials of One-step Digestion Buffer; add 1.3 mL of deionized water to each vial before use to ensure complete dissolution.

2. Use 1.3 mL*2 mL (when using 48x kit) or 0.5 mL (when using 8x kit) of **One-step Digestion Buffer** to fully dissolve the **Enzyme**.

3. Add 50 µL dissolved **Enzyme** to the microcentrifuge tube containing **Enrichment Nanobeads** from the last step. Incubate and shake at 1500 rpm at 45 °C on microcentrifuge tube heater shaker for 1 hours OR at 37 °C for overnight.

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 **EV Enrichment Nanobeads** is

completely collected by the magnet. Transfer the supernatant (approximately 120 µL) by pipetting for subsequent of processing.

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
REF	Catalogue number		Keep away from sunlight
LOT	Batch code		Consult instructions for use
	Temperature limit: 2~8°C		Date of manufacture
	Use-by date		Manufacturer
CE	CE mark		

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EC REP

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