Nanotrap Protein Enrichment Affinity Kit User Guide

Manual Protein Enrichment from Plasma Samples Collected in K2EDTA Blood Collection Tube or Streck Protein Plus BCT

This protocol describes a manual method using the Nanotrap® Protein Enrichment Affinity Kit (PEAK) to enrich low abundance proteins and peptides in plasma collected in K2EDTA Blood Collection Tube or Streck Protein Plus BCT™.

Kit Contents and Equipment Lists

Download the Kit Contents and Equipment Lists PDF at https://bit.ly/peak_user_guide

Troubleshooting

- Ensure that all reagents are stored at the indicated temperatures.
- Make sure refrigerated reagents and plasma samples (if applicable) are warmed to room temperature before starting the procedure.
- If you are interested in using alternate reduction, alkylation, and digestion steps, please contact support@ceresnano.com for technical assistance.
- Nanotrap® Protein Particles must be adequately vortexed immediately before each use.
- Add the reagents in the order listed in the corresponding procedure.
  - Do not mix Nanotrap® Protein A Particles, Nanotrap® Protein B Particles, and Nanotrap® Protein C Particles directly with each other prior to adding them to the diluted plasma sample.

Kit Guidelines

- **Reagent Preparation**
  - **Preparation of 5% TFA. Make a fresh preparation every day.**
    - In a 2 mL microcentrifuge tube, add 5 µL of 99% TFA to 95 µL of HPLC grade water. Vortex thoroughly to mix.
  - **Preparation of 50 mM Ammonium Bicarbonate, pH 7.8 – 8.2. Ensure pH is within the working range of 7.8 – 8.2 before using.**
    - To a 50 mL conical tube, or similar container, add 0.197 g of Ammonium Bicarbonate.
    - Bring the volume up to 50 mL using deionized water. Vortex thoroughly until the solution is completely clear and Ammonium Bicarbonate is dissolved.
    - Using a calibrated benchtop pH meter, confirm that the Ammonium Bicarbonate solution is within the working pH range of 7.8 – 8.2.
- **Unless otherwise indicated, perform all steps in the protocols at room temperature (15°C to 30°C).**
  - Allow Nanotrap Protein Particles and plasma sample(s) to reach room temperature.
    ~If plasma sample is frozen, it may be placed at up to 37°C until fully thawed.

- **Nanotrap Protein Particles must be vortexed for at least 30 seconds prior to use.**
- **Selecting the Nanotrap Protein Particle combination:**
  - This protocol can be used with Nanotrap Protein A Particles, Nanotrap Protein B Particles, Nanotrap Protein C Particles, or with all three Nanotrap Protein Particles with a 50 µL plasma sample collected in a K2EDTA blood collection tube or a Streck Protein Plus BCT. For each 50 µL plasma sample, choose one of the following:
    – 200 µL of Nanotrap Protein A Particles plus 150 µL of Nanotrap® Buffer 4; or
    – 200 µL of Nanotrap Protein B Particles plus 150 µL of Nanotrap Buffer 4; or
    – 200 µL of Nanotrap Protein C Particles plus 150 µL of Nanotrap Buffer 4; or
    – 66 µL each of Nanotrap Protein A Particles, Nanotrap Protein B Particles, and Nanotrap Protein C Particles plus 150 µL of Nanotrap Buffer 4.

Preparation of Plasma from Whole Blood from Streck Protein Plus BCT

Prepare plasma as recommended by tube manufacturer:

1. Centrifuge whole blood samples at 1,800 x g for 15 minutes at room temperature.
2. Carefully transfer plasma to a new tube.
3. Centrifuge the plasma at 2,800 x g for 15 minutes at room temperature.
4. Carefully transfer plasma to a new tube (or directly to sample tubes).

Note: After transferring plasma samples to a new tube, see manufacturer guidelines for recommended plasma storage.

Preparation of Plasma from Whole Blood from K2EDTA Blood Collection Tubes

Prepare plasma as recommended by tube manufacturer:

1. Centrifuge whole blood samples at 1,600 x g for 10 minutes at room temperature.
2. Carefully transfer plasma to a new tube.

Note: After transferring plasma samples to a new tube, see manufacturer guidelines for recommended plasma storage.

Experimental Setup

1. Preheat the heat block to 37°C.

Protein Enrichment

Centrifuge plasma samples at 5,000 x g for 2 minutes.

In a 2 mL microcentrifuge tube, complete in order:

1. Add 50 µL of plasma to the sample tube.
2. Add 150 µL Nanotrap Buffer 4 to the sample tube.
3. Vortex Nanotrap Protein Particles for 30 seconds to resuspend.
4. Add 200 µL of the Nanotrap Protein Particle type of your choice to each sample tube.
   a) ***Alternatively, you can use all three Nanotrap Protein Particles in a single sample by following these steps:***
   b) Add 66 µL of Nanotrap Protein Particle A into the sample tube and vortex for 30 seconds at room temperature using a vortex mixer.
   c) Add 66 µL of Nanotrap Protein Particle B to the sample tube. Vortex for 30 seconds at room temperature using a vortex mixer.
   d) Add 66 µL of Nanotrap Protein Particle C to the sample tube.
5. Vortex sample tube for 30 seconds to ensure that the Nanotrap Protein Particles are fully resuspended and incubate for 30 minutes at room temperature while shaking on a vortex mixer at medium speed.
6. Place the sample tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles. While the sample tube is still on the magnetic rack, remove the supernatant and discard.
7. Add 500 µL deionized water to the Nanotrap Protein Particle pellet and vortex for 30 seconds to ensure the Nanotrap Protein Particles are fully resuspended.
8. If needed, briefly centrifuge the sample tube for 5 seconds to pull down any water droplets from the side or cap of the microcentrifuge tube.
9. Place the sample tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles. While the sample tube is still on the magnetic rack, remove the supernatent and discard.
10. Repeat steps 7, 8 and 9 one time each for a total of two deionized water washes.

Protein Digestion

If you are interested in using alternate reduction, alkylation, and digestion steps, please contact support@ceresnano.com for technical assistance.

1. Add 100 µL of 50 mM Ammonium Bicarbonate (pH 7.8 - 8.2) to the Nanotrap Protein Particles pellet.
2. Vortex the sample tube for 30 seconds to ensure that the Nanotrap Protein Particles are fully resuspended.
3. Add 1 µL of 500 mM TCEP to the sample tube and vortex for 30 seconds to ensure the sample is mixed.
4. If needed, briefly centrifuge the sample tube for 5 seconds to pull down any droplets from the side or cap of the microcentrifuge tube.
5. Place the sample tube in the heat block at 37°C for 30 minutes.
6. Remove the sample tube from the heat block and allow the sample tube to cool to room temperature.

7. Immediately before use, dissolve one tube of iodoacetamide (9.3 mg) with 132 µL of 50 mM Ammonium Bicarbonate to make 375 mM iodoacetamide.
8. Add 5 µL of the 375 mM iodoacetamide to the sample tube, **vortex for 30 seconds at room temperature using a vortex mixer.** Incubate for 30 minutes at room temperature protected from light.
9. While the sample is incubating at room temperature, preheat the heat block to 70°C.
10. Place the sample tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles. Remove the supernatant and discard.
11. Add 500 µL of 50 mM Ammonium Bicarbonate to the Nanotrap Protein Particle pellet and vortex for 30 seconds to ensure the Nanotrap Protein Particles are fully resuspended.
12. Place the sample tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles. Remove the supernatant and discard.
13. Add 500 µL deionized water to the Nanotrap Protein Particle pellet and vortex for 30 seconds to ensure the Nanotrap Protein Particles are fully resuspended.
14. If needed, briefly centrifuge the sample tube for 5 seconds to pull down any water droplets from the side or the cap of the microcentrifuge tube.
15. Place the sample tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles. Remove the supernatant and discard.
16. Repeat steps 13, 14, and 15 one time each for a total of two deionized water washes.
17. Add 10 µL deionized water and 35 µL of Promega Rapid Digest Buffer to the Nanotrap Protein Particles pellet and vortex for 30 seconds to ensure the Nanotrap Protein Particles are fully resuspended.
18. Resuspend 100 µg of the Promega Rapid Trypsin/Lys-C Mix in 100 µL of Promega Resuspension Buffer to a final concentration of 1 mg/mL.
19. Add 5 µL of the 1 mg/mL Rapid Trypsin/Lys-C Mix to each sample tube and vortex for 5 seconds to ensure the Nanotrap Protein Particles are fully resuspended.
20. Briefly centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
21. Place the sample tube in the heat block at 70°C for 2 hours.
22. Remove sample tube from the heat block and let sample tube cool for 2 minutes at ambient temperature.
23. Centrifuge the sample tube for 5 seconds to bring down condensation vapors collected on the tube cap and sides.
24. Following centrifugation, add 2 µL of 5% TFA to the reaction to terminate it and vortex for 5 seconds.
25. Place the tube on the magnetic rack for 30 seconds to pellet the Nanotrap Protein Particles.
26. Transfer the supernatant to a new 2 mL microcentrifuge tube.
27. Store samples at -20°C or below or proceed directly to desalting and LC-MS/MS.

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