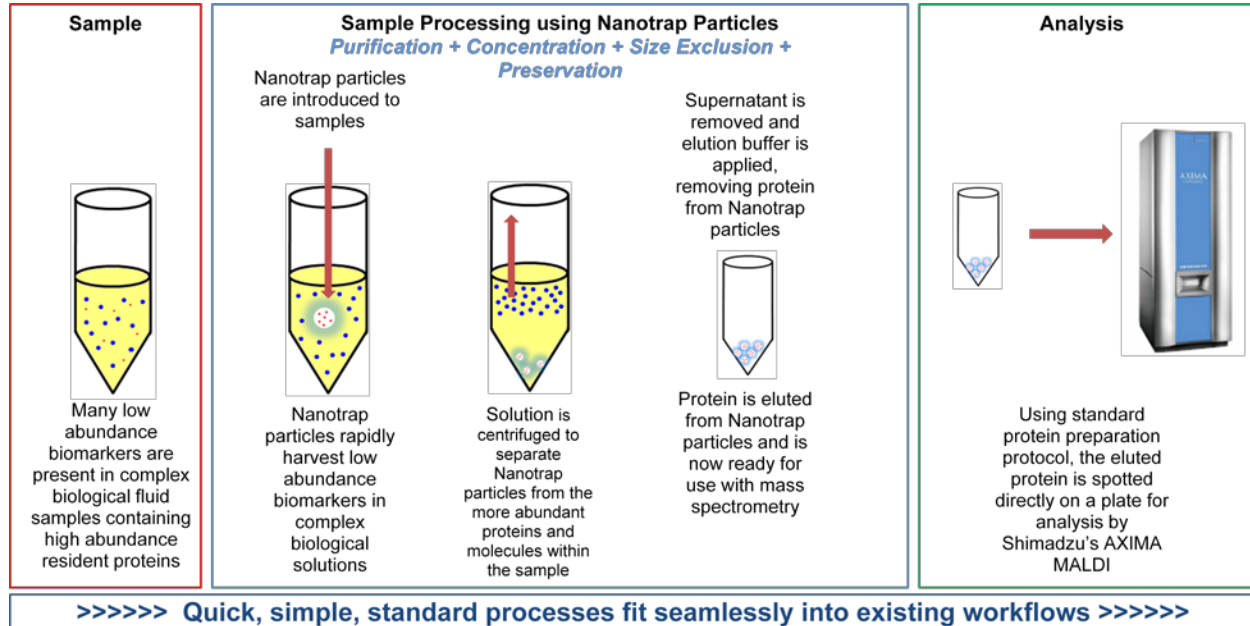


# Nanotrap<sup>®</sup> Biomarker Discovery Platform

## Nanotrap workflow for serum samples

This kit contains Nanotrap<sup>®</sup> particles for sample preparation, fractionation and pre-concentration prior to use with Shimadzu AXIMA MALDI Mass Spectrometers and Progenesis MALDI bioinformatics software. Nanotrap<sup>®</sup> particles are designed for the fractionation and concentration of low abundance proteins, peptides and protein conjugates (analytes) from complex biofluid matrices. **This protocol is specifically designed for serum and plasma sample preparation prior to MALDI analysis. Please contact [support@ceresnano.com](mailto:support@ceresnano.com) for specific protocols optimized for alternative sample types and analysis methods.**



### 1.0 Description

The Nanotrap<sup>®</sup> Biomarker Discovery Platform provides a simple, rapid and reliable method to purify, concentrate and prevent degradation to proteins, peptides and low molecular weight compounds found in complex biological matrices. Nanotrap<sup>®</sup> particles are designed to harvest proteins and other desired molecules directly from biological matrices including serum, plasma, cell culture supernatant and urine.

Nanotrap<sup>®</sup> particles do not rely on specific antibodies, which allows them to fractionate, concentrate and protect a range of proteins and peptides prior to identification and quantification using Shimadzu mass spectrometers. The ability to trap and harvest a series of protein or peptides based on tailored affinity and size characteristics at the same time improves work flow, while reducing both required sample volumes and processing time.

### 2.0 Product Components and Storage Conditions

1. Nanotrap<sup>®</sup> particles - Store at room temperature, do not freeze.

#### Additional Reagents, Materials and Equipment Required

1. Elution Buffer (70% Acetonitrile, 10% Ammonium hydroxide)
2. Sodium thiocyanate (NaSCN)
3. 18.0 MΩ-cm purified water
4. Micro-centrifuge (14,800 rpm/21,100 g)
5. Shimadzu Mass Spectrometer
6. Vacuum concentrator

### 3.0 Protocol

This procedure provides a method for the application of Nanotrap<sup>®</sup> particles designed to harvest, protect and concentrate low to medium molecular weight proteins and peptides in serum samples. Note: Recommended sample volume is dependent on the protein of interest. 50 µl of sample is suggested for serum, but higher/lower volumes may be necessary.

#### Harvest

1. Pipette 100 µl of Nanotrap<sup>®</sup> particles into a micro-centrifuge tube.
2. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.

3. Carefully remove and discard the supernatant wash without disturbing the pellet.
4. Resuspend the particles in 100 µl of 18 MΩ-cm water to wash.
5. Pipette the washed particles into a micro-centrifuge tube containing the sample.
6. Suspend the particles within the biological sample matrix and allow the particles to harvest the desired analytes for 30 minutes at room temperature.

#### Spin

7. Centrifuge the particle - biological fluid suspension at 14,800 rpm/21,100 g for 7 minutes.
8. Remove the supernatant and transfer to a new micro-centrifuge tube if downstream analysis of the supernatant is desired; otherwise discard supernatant. (Note: It may be difficult to see the pellet of White Nanotrap<sup>®</sup> particles, try viewing pellet against a light source.)

#### Wash

9. Resuspend blue particles in 100 µL of 0.5M Sodium thiocyanate to wash. **White** particles should be washed in 100 µl of 18 MΩ-cm water.
10. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.
11. Repeat Steps 9 and 10.
12. Resuspend the particles in 100 µl of 18 MΩ-cm water to wash.
13. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.
14. Repeat Steps 12 and 13.
15. Prepare fresh elution buffer (70% Acetonitrile, 10% Ammonium hydroxide). Note: For best results, elution buffer must be prepared fresh every time.
15. Carefully remove and discard the supernatant wash without disturbing the pellet.

#### Elution

16. Resuspend the particles in 20 µl of elution buffer and incubate for 15 minutes at room temperature.
17. Centrifuge the particles at 14,800 rpm/21,100 g for 15 minutes.

18. Carefully remove and save the supernatant (elution).
19. Use a vacuum concentrator to evaporate the elution.
20. Reconstitute the sample with matrix (alpha-cyano-4-hydroxycinnamic acid (4 mg/ml) in 50% ACN/50% H<sub>2</sub>O, 0.1% TFA). Reconstitution volumes will vary depending on the protein concentration of the sample, suggested volume is 50 µl.
21. Allow sample to remain in matrix for 5 minutes before spotting the plate.
22. Spot 0.5 µl of your diluted sample onto a MALDI plate for analysis.

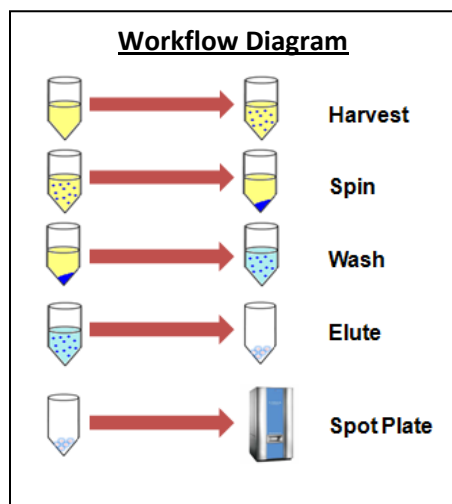
#### **4.0 Trouble Shooting**

Nanotrap<sup>®</sup> particles are not forming a pellet or forming a loose pellet.

- Centrifuge the particles at a higher speed or for a longer duration.

Few or no proteins are showing up in the Nanotrap<sup>®</sup> particles.

- Determine that the sample solution contains a concentration of protein high enough for the chosen analysis technique; increase the starting sample volume into which the Nanotrap<sup>®</sup> particles are added.



For further troubleshooting, visit our support site at [www.ceresnano.com](http://www.ceresnano.com).

#### **5.0 Product use**

Nanotrap<sup>®</sup> particles are manufactured by Ceres Nanosciences, LLLP ("Ceres"). This product conforms to specifications indicated for the intended use.

#### **Warranty**

Ceres does not guarantee the performance of our particle technology for specific applications. Nanotrap<sup>®</sup> particles conform to physical and performance criteria for gel electrophoresis sample processing for the duration of the stated shelf life. Ceres' obligation under this warranty is limited to replacement, at Ceres' expense, of any product which is deemed defective in manufacture. Defective product must be returned to Ceres with proof of such defect. Claims resulting from merchandise damaged during shipping and delivery should be directed to the carrier. This warranty does not apply to any products that have been altered, improperly stored or misused. ALL OTHER WARRANTIES, EXPRESSED, IMPLIED OR STATUTORY, ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Ceres' maximum liability is limited in all events to the price of the products sold by Ceres in each instance of a claim. IN NO EVENT SHALL CERES NANOSCIENCES BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow limits on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply, however such limits as otherwise codified by such state law are hereby incorporated by reference to the maximum benefit of such disclaimer on behalf of Ceres.

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#### **Intellectual Property Disclaimer**

Ceres will not be responsible for violations or patent infringements that may occur with the use of our products

#### **Limited use statement**

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