



# Nanotrap Biomarker Discovery Platform

Protocols for Biofluid Sample Preparation and Analyte Enrichment

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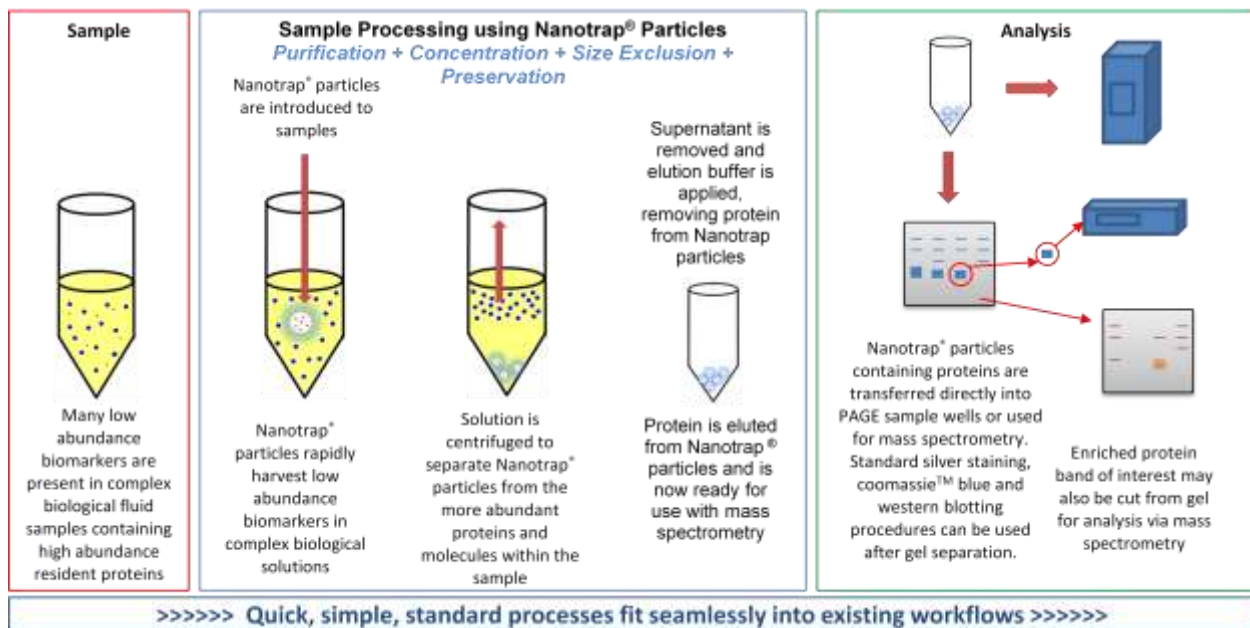
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## Introduction

This kit contains Nanotrap<sup>®</sup> particles for sample preparation, fractionation and pre-concentration prior to use with sample analysis methods. Nanotrap<sup>®</sup> particles are designed for the fractionation and concentration of low abundance proteins, peptides and protein conjugates (analytes) from complex biofluid matrices. The user manual contains a protocol specifically designed for sample preprocessing prior to analysis on Shimadzu Axima MALDIs as well as protocols for sample fractionation and enrichment prior to gel electrophoresis and other Mass Spectrometry analysis methods. **Please contact [support@ceresnano.com](mailto:support@ceresnano.com) for additional protocols optimized for alternative sample types and analysis methods.**

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, saliva, cerebrospinal fluid, cell culture supernatant and urine. Nanotrap<sup>®</sup> particles do not rely on specific antibodies, which allow them to fractionate, concentrate and protect a range of proteins and peptides prior to identification and quantification using mass spectrometry, gel electrophoresis or other immunoassays. 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.



## Product Components and Storage Conditions

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

## Materials Supplied by the User

### Mass Spectroscopy Protocols

1. Biofluid Sample
2. Elution Buffer-Blue (70% Acetonitrile, 10% Ammonium hydroxide)
3. Elution Buffer-White(60% Acetonitrile, 2% Acetic Acid)
4. Sodium thiocyanate (NaSCN)
5. 18.0 MΩ-cm ultrapure water
6. Micro-centrifuge tubes
7. Pipettors and tips
8. Micro-centrifuge (14,800 rpm/21,100 g)
9. Vacuum concentrator
10. Shimadzu Mass Spectrometer

### Gel Electrophoresis Protocols

1. Biofluid Sample
2. Sodium thiocyanate (NaSCN)
3. 18.0 MΩ-cm ultrapure water
4. Micro-centrifuge (14,800 rpm/21,100 g)
5. Micro-centrifuge tubes
6. Pipettors and tips
7. Vacuum concentrator
8. Gel electrophoresis box
9. Elution Buffer-Blue (70% Acetonitrile, 10% Ammonium hydroxide) – only required for elution procedure
10. Elution Buffer-White(60% Acetonitrile, 2% Acetic Acid) – only required for elution procedure

## Nanotrap<sup>®</sup> Workflow for MALDI Analysis

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.

### 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 (Blue - 70% Acetonitrile, 10% Ammonium hydroxide, White - 60% Acetonitrile, 2% Acetic Acid). Note: For best results, elution buffer must be prepared fresh every time.
16. Carefully remove and discard the supernatant wash without disturbing the pellet.

#### Elution

17. Resuspend the particles in 20 µl of elution buffer and incubate for 15 minutes at room temperature.
18. Centrifuge the particles at 14,800 rpm/21,100 g for 15 minutes.
19. Carefully remove and save the supernatant (elution).
20. Use a vacuum concentrator to evaporate the elution.
21. Reconstitute the sample with matrix (alpha-cyano-4-hydroxycinnamic acid (10 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.
22. Allow sample to remain in matrix for 5 minutes before spotting the plate.
23. Spot 0.5 µl of your diluted sample onto a MALDI plate for analysis.



## Nanotrap<sup>®</sup> Workflow for Mass Spectrometry Analysis

This protocol is specifically designed for serum and plasma sample preparation. Please contact [support@ceresnano.com](mailto:support@ceresnano.com) for specific protocols optimized for alternative sample types and analysis methods.

### 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. 50µl 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 75 µl of 18 MΩ-cm water to wash.
5. Pipette the washed particles into a micro-centrifuge tube containing the biofluid 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.
7. Prepare elution buffer. (Note: For best results, elution buffer must be prepared fresh every time.)

#### Spin

8. Centrifuge the particle - biological fluid suspension at 14,800 rpm/21,100 g for 7 minutes.
9. 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

10. 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.
11. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.
12. Repeat Steps 9 and 10.
13. Resuspend the particles in 100 µl of 18 MΩ-cm water to wash.
14. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.
15. Repeat Steps 12 and 13.
16. Prepare fresh elution buffer Blue - (70% Acetonitrile, 10% Ammonium hydroxide), White- (60% Acetonitrile, 2% Acetic Acid). Note: For best results, elution buffer must be prepared fresh every time.
24. Carefully remove and discard the supernatant wash without disturbing the pellet.

#### Elution

17. Resuspend the particles in 20µl of elution buffer and incubate for 15 minutes at room temperature.
18. Centrifuge the particles at 14,800 rpm/21,100 g for 15 minutes.
19. Carefully remove and save the supernatant (elution).
20. Use a vacuum concentrator to evaporate the eluate and resuspend analytes in 5 µl of water. (Note: Dilutions of analytes may be required.)
21. Proceed with desired method of analysis.



## Nanotrap<sup>®</sup> Workflow for Gel Electrophoresis – Electro-Elution

This protocol is specifically designed for serum and plasma sample preparation prior to gel electrophoresis. Particles containing harvested analytes are loaded directly into your gel. Please contact [support@ceresnano.com](mailto:support@ceresnano.com) for specific protocols optimized for alternative sample types and analysis methods.

### 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. 20µl of sample is suggested for serum or plasma, 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 18.0 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.

#### Elution

15. Resuspend the particles in 20 µl of 2x SDS sample buffer. Heat the prepared particles for approximately 5 minutes at 100°C.
16. Carefully, load the gel with the remaining sample buffer.

## Nanotrap® Workflow for Gel Electrophoresis – Elution Prior to SDS-PAGE Separation

This protocol is specifically designed for serum and plasma sample preparation prior to SDS -PAGE. This protocol requires analytes to be eluted from particles prior loading gel. Please contact [support@ceresnano.com](mailto:support@ceresnano.com) for specific protocols optimized for alternative sample types and analysis methods.

### Protocol

This procedure provides a method for the application of Nanotrap® 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.** 20µl of sample is suggested for serum or plasma, but higher/lower volumes may be necessary.

#### Harvest

1. Pipette 100 µl of **Nanotrap® 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. For recommended sample volume see note above.
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® 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 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 Blue-(70% Acetonitrile, 10% Ammonium hydroxide), White-(60% Acetonitrile, 2% Acetic Acid). Note: For best results, elution buffer must be prepared fresh every time.
25. Carefully remove and discard the supernatant wash without disturbing the pellet.

#### Elution

16. Resuspend the particles in 20µl of elution buffer and incubate for 15 minutes at room temperature.
17. Centrifuge the particles at 14,800 rpm/21,100 g for 7 minutes.
18. Carefully remove and save the supernatant (elution).
19. Add elution to 15 µL of 2x SDS sample buffer. Heat the prepared particles for approximately 10 minutes at 100°C. The microcentrifuge tubes should be uncovered to allow the elution buffer to evaporate.
20. Load the gel with the remaining sample buffer.

## Technical Support

Visit the Ceres Nano website at [www.ceresnano.com/support](http://www.ceresnano.com/support) for:

- Technical resources, including product inserts, FAQs, application notes, publications, MSDSs, etc.
- Technical support contact information
- Additional product information

## Contact Us

For more information or technical assistance, please call or email.

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Material Safety Data Sheets (MSDSs) are available on our website at [www.ceresnano.com/msds](http://www.ceresnano.com/msds).

## Product Use Statement

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.

### Patents and trademarks

“Nanotrap” is a trademark of Ceres. The Nanotrap particles are the subject of numerous United States and foreign patent applications. Any registration or trademark symbols used herein denote the registration status of trademarks in the United States.

### 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

The purchaser of this product has the non-transferable right to use Nanotrap<sup>®</sup> Technology for Research conducted solely by the purchaser. The buyer cannot sell or otherwise transfer this product or materials made using this product to a third party or otherwise use this product or materials made using this product for Commercial Purposes. The buyer may transfer information created through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing not to transfer such materials to any third party, and to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, prophylactic or other similar or related purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Ceres will not assert a claim against the buyer for infringement of patents owned or controlled by Ceres which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Ceres is willing to accept return of the product, FOB Ceres, with a full refund, provided the product has not been opened or otherwise used. Any use or the opening of the product package automatically obligates the purchaser to the terms and conditions herein and a non-refundable purchase of product. For information on purchasing a license to this product for purposes other than research, contact: [info@ceresnano.com](mailto:info@ceresnano.com) tel: 1-800-615-0418.



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