Nanotrap® Virus Capture Kit User Manual

For the capture and concentration of viruses from complex samples

Product SKUs 44250, 44210, 44201, 44202

Protocol: APP-UM-003; V:2; Release Date: 7 February 2020

Nanotrap® Virus Capture Kit

Intended Use

This kit can be used to capture and concentrate viruses from transport media, cell culture media, urine, cerebrospinal fluid, diluted plasma, and diluted serum. Captured viruses can be used in a variety of downstream analyses, including nucleic acid analysis, protein analysis, and viral infectivity assays. For a full list of virus applications that users have published using Nanotrap® Virus Particles, visit https://www.ceresnano.com/viruscapture. For recommendations on how to apply this kit for other viruses, sample types, and analysis methods, contact *support@ceresnano.com*.

This document must be read in its entirety before using this product.

FOR RESEARCH USE ONLY

Nanotrap® kits are not intended or validated for use in the diagnosis of disease or other conditions.

Ceres Nanosciences

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Contact Information

For information regarding Ceres Nanosciences' products, for technical support, or for general inquiries, please contact:

Ceres Support

Phone: +1-800-615-0418
Email: *support@ceresnano.com*

Introduction

The Nanotrap® Virus Capture Kit contains Nanotrap® Lyophilized Magnetic Virus Particles. It can be used to capture and concentrate viruses found in complex samples. The following protocol has been confirmed for the capture and concentration of influenza virus from transport media samples upstream of a QIAGEN nucleic acid extraction kit from (QIAGEN Catalog # 52904) and a glycogen nucleic acid precipitation method prior to qRT-PCR analysis. The Nanotrap® Virus Capture Kit can be adapted for other virus types, samples, and assays. For a full list of applications that users have published using Nanotrap® particles, visit https://www.ceresnano.com/viruscapture. Nanotrap® Virus Particles are available in non-magnetic and magnetic versions. Magnetic and non-magnetic versions of these particles may have different virus binding profiles. Bulk purchasing options are also available. If you would like to try non-magnetic Nanotrap® Virus Particles or discuss bulk purchasing, please contact *support@ceresnano.com.*

Principle of Nanotrap® Technology

Nanotrap® particles are hydrogel particles made of cross-linked N-isopropylacrylamide (NIPAm) polymers functionalized with chemical affinity baits. Nanotrap® particles have large surface areas and are highly porous, both of which facilitate high binding capacity and rapid binding. The chemical affinity baits that are used in Nanotrap® particles are selected for their ability to bind, with very high affinity, to different classes of analytes. These baits bind to their targets primarily through electrostatic and hydrophobic interactions.[1] See Figure 1 for more details on the chemistry and architecture of Nanotrap® particles.

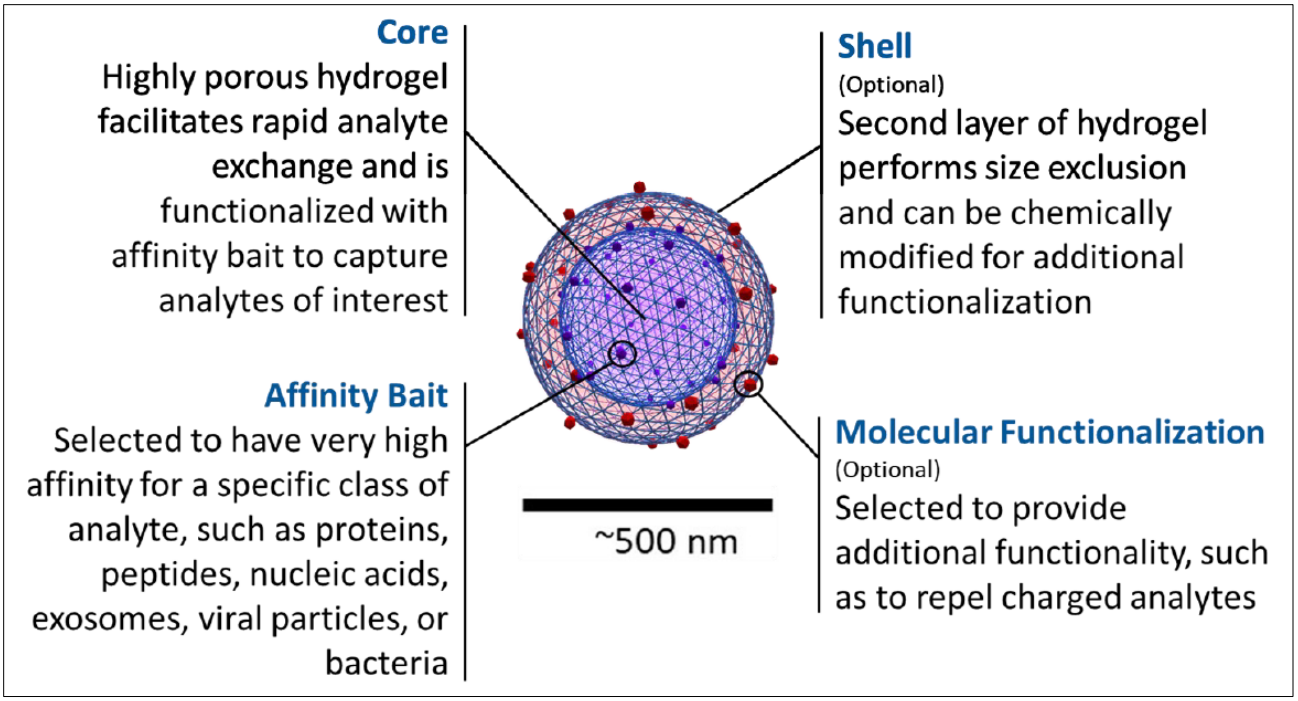

Figure 1: Nanotrap® particles can be customized for different applications

Ceres Nanosciences has many available particle types that can be optimized for different applications and workflows. Nanotrap® core particles are used in applications

[1] Bancroft JD, Gamble M, ed. (2002), *Theory and Practice of Histological Techniques*. 5th ed. London: Churchill-Livingstone. ISBN 0443064350.

where the target analyte is large, such as a virus, bacteria, or extracellular vesicle. Nanotrap® core-shell particles are used in applications where the target analyte is small. The shell structure is used to restrict access of high-molecular weight molecules to the affinity bait in the core of the particles. Both core and core-shell particles can be magnetically functionalized for applications where magnetic processing is desired. Core, core-shell, and magnetically functionalized Nanotrap® particles can be lyophilized. Nanotrap® particles are effective at capturing and concentrating analytes of interest from a wide variety of sample types prior to downstream analysis. To learn more, visit our applications page: https://www.ceresnano.com/literature.

Nanotrap® Virus Capture Particles Workflow

1. Capture
    - Nanotrap® particles (magnetic or non-magnetic) are mixed with the sample and capture viral particles.
    - Nanotrap® particles and captured viruses are separated from the rest of the sample with a magnet or with a centrifuge.
    - The supernatant is removed from the Nanotrap® particles and the captured viruses.
2. Extract
    - Nanotrap® particles and captured viruses are incubated in the appropriate volume of extraction buffer.
    - Nanotrap® particles are separated from the extraction buffer, which contains viral material, with a magnet or with a centrifuge.
3. Analyze
    - Extracted proteins or nucleic acids are removed from the Nanotrap® particles and are further processed or are used for downstream analysis.

Kit Components

Kit Components	Units
Nanotrap® Lyophilized Magnetic Virus Particles*	50 x 0.2 mL (# 44250) or 10 x 0.2 mL (# 44210)

*Nanotrap® Lyophilized Magnetic Virus Particles are provided as lyophilized, single-use aliquots and should be stored between 15 and 25 degrees Celsius.

Recommended Materials

- Deionized Water
- 50 mM Tris-HCl, pH 7.4, for diluting serum or plasma samples (For example, Sigma-Aldrich Catalog # T2194)
- Vortex, tube rotator, or shaker (For example, Scilogex Vortex,

  Scilogex Catalog # 821200049999)
- Magnetic separator (For example, DynaMag-2 mL Magnetic Rack, ThermoFisher Catalog # 12321D)
- Microcentrifuge that can reach speeds of 21,000 RCF, for separating Nanotrap® particles from samples if you do not plan to use magnetic separation (For example, Sorvall Legend Micro21,
  ThermoFisher Catalog # 75002437)

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples and reagents properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the material safety data sheet (MSDS), which can be obtained from Ceres Nanosciences at *info@ceresnano.com*.

Quality Control

In accordance with Ceres Nanosciences' Quality Management System, each lot of Nanotrap® Lyophilized Magnetic Particles is tested against predetermined specifications.

Sample Preparation

For native or contrived samples, the following volume ranges and dilution factors are recommended in the table below by sample type.

Sample type	Volume	Dilution
Plasma	Up to 2 mL	Dilute 1:1 with 50 mM Tris HCl (up to 4 mL total)
Serum	Up to 2 mL	Dilute 1:1 with 50 mM Tris HCl (up to 4 mL total)
Cell culture media	Up to 5 mL	Dilution not required
Transport media	Up to 3 mL	Dilution not required
CSF	Up to 500 µL	Dilution not required
Urine	Up to 5 mL	Dilution not required

Table 1. Sample type, volume, and sample dilution recommendations.

Sample volumes may be increased or decreased to optimize virus capture and concentration.

Best Practices

Sample Preparation

Sample centrifugation - Optional

- Visually inspect each sample to be analyzed. If the sample appears turbid, centrifuge at 5,000 – 21,000 RCF for 2 minutes to remove aggregates or debris prior to adding Nanotrap® particles.

Using Nanotrap® Particles

Capture

- Add your sample to the tube containing the Nanotrap® particles to ensure homogenous particle suspension after sample addition.

Supernatant removal from Nanotrap® particles

- When Nanotrap® particles are pelleted, carefully pipette off the supernatant from the side opposite the particle pellet to avoid risk of aspirating the Nanotrap® particles. Video *here*.

Nanotrap® particle resuspension

- To resuspend the particle pellet efficiently, pipette the solution up and down to break up pellet into smaller clumps. Continue to pipette the suspension until the pellet is completely resuspended. The particles can be vortexed to mix after the pellet is resuspended.
- Do not use the pipette tip to poke at the pellet in an attempt to break it up, as the Nanotrap® particles may stick to the tip. If Nanotrap® particles stick to the tip, carefully scrape the pellet off the tip by scraping the tip on the lip of the tube. Then pipette liquid onto the particle pellet and pull pellet down with magnet or by centrifuging the tube.
- Avoid introducing air bubbles into the solution as this may cause foaming.

Protocol

This procedure provides a general method for the virus enrichment with Nanotrap® Lyophilized Magnetic Virus Particles.

Capture

1. Add up to 1.9 mL of sample (minimum of 0.2 mL) to a single tube of Nanotrap® Lyophilized Magnetic Virus Particles. Recap and vortex at high speed for five seconds to mix. See Table 1 for sample volumes and diluents. The Nanotrap® particle tube can hold up to 2 mL of sample. If you plan to use more than that volume of sample, you can resuspend the lyophilized Nanotrap® particles in 200 µL of deionized water and transfer the resuspended Nanotrap® particles to a larger tube with your sample in it.
    a. **Note: Increasing the starting sample volume can increase the enrichment of certain viruses. See Table 1 for maximum sample volumes for different sample types. Please contact support@ceresnano.com for more information.**
    b. **Note: Increasing the dilution factor for the starting sample, especially if using serum and plasma, can improve capture efficiency of certain viruses. See Table 1 for more details.**
2. Allow the Nanotrap® particles to incubate in the sample for 20 minutes at room temperature, with constant rotation or gentle agitation. *Alternatively, samples may be mixed manually every 5 minutes.*
    a. **Note: Capture incubation times can be lengthened or shortened to optimize and streamline workflow while ensuring efficient capture of viruses. Users can evaluate capture incubation times between 2 and 30 minutes.**
3. Place tubes in a magnetic rack and allow 1-2 minutes for separation. It may take longer to pellet the Nanotrap® particles in larger volumes. Without disturbing the Nanotrap® particle pellet, remove the supernatant following *Best Practices.*

Extract

4. Refer to your downstream extraction kit protocol and resuspend the pellet with the Nanotrap® particles and the captured viruses in the appropriate volume of the required buffer to begin that protocol following *Best Practices*. For example:
    a. If you are using a viral RNA extraction kit that calls for a starting sample volume of 140 µL to which you add 560 µL of lysis buffer, then resuspend the Nanotrap particles in

140 µL of 1X PBS and add 560 µL of lysis buffer and incubate for an appropriate amount of time, as described in the extraction kit user manual.

b. If you are using a glycogen nucleic acid precipitation method, resuspend the Nanotrap® particle pellet in 100 µL of an appropriate lysis buffer and incubate for 10 minutes at room temperature.

c. If you plan on running a Western Blot on the concentrated virus, then resuspend your Nanotrap® particle pellet in the appropriate volume of Laemmli buffer and boil the sample at 95 degrees Celsius for 3-5 minutes.

d. If you are interested in another type of downstream assay, please visit https://www.ceresnano.com/viruscapture for additional information, or contact Ceres Nanosciences at info@ceresnano.com with your question.

5. Separate the depleted Nanotrap® particles from your extracted sample by placing tubes in a magnetic rack for 1-2 minutes. It may take longer to pellet the Nanotrap® particles in larger volumes. Without disturbing the Nanotrap® particle pellet, transfer the extracted sample into a clean tube or well plate following *Best Practices.*
6. Proceed with your downstream protocol using the extracted sample.

Note: If you prefer not to use magnetic separations, both magnetic and non-magnetic Nanotrap® particles can be separated from the supernatant using centrifugation. For centrifugation conditions for Nanotrap® particles, see the Troubleshooting Guide below.

Troubleshooting Guide

We love to help! The scientists at Ceres are always happy to answer any questions you may have about either the information and/or protocols in this user manual. For more information visit www.ceresnano.com/contact-us.

ISSUE	SOLUTION
The Nanotrap® particles do not pellet after the magnetic separation step.	Place the magnet closer to the sample and/or increase the amount of time that the magnet is in contact with the sample.
The Nanotrap® particles do not pellet after the magnetic separation step.	If you have access to a centrifuge that can reach high speeds, centrifuge your samples for 10 minutes at a minimum of 16,800 RCF. ***The centrifuge must be able to reach a maximum speed of at least 16,800 RCF.***

	1. If a diffuse pellet forms or the pellet is not visible after centrifugation, spin the samples for an additional 2 – 7 minutes at 16,800 RCF or increase the centrifuge speed to 21,100 RCF. 2. If using a sample volume greater than 1.00 mL, increase the centrifugation time to 15 – 20 minutes at 16,800 RCF.
Particles are not resuspending well in my extraction buffer.	After attempting to resuspend particles following *Best Practices*, try vortexing particles to resuspend.
There are contaminating molecules in my sample after I capture and concentrate my viruses with Nanotrap® particles.	Particle washing: Prior to resuspending the Nanotrap® particles and captured virus in your extraction buffer, the virus-bound Nanotrap® particles can be washed one, two, or three times with 1 mL of 18 MΩ-cm water to remove any contaminating proteins or molecules.
The target analyte is undetectable in my downstream assay.	Determine if the concentration of the target analyte is within the detection limit of the given method of analysis. 1. If the starting concentration of the target analyte is lower than the method's detection limit: a. Use a more sensitive detection method b. Increase the starting sample volume c. Start with a higher concentration of your target analyte 2. If the starting concentration is within the method's detection limit: a. Run the sample supernatant from the initial incubation with Nanotrap® particles on the downstream assay to determine if the analyte has NOT been captured by the Nanotrap® particles. If the target analyte is found in the supernatant and was not captured by the Nanotrap® particles, contact *support@ceresnano.com* for support. For a full list of virus types that users have published using Nanotrap® Virus Particles, visit https://www.ceresnano.com/viruscapture.

Terms & Conditions

Product Use

Nanotrap® particles are manufactured by Ceres Nanosciences, Inc. ("Ceres"). This product conforms to specifications indicated for the intended use. (See complete terms at http://www.ceresnano.com/ceres-terms-of-sales-and-use .)

Warranty

Ceres does not guarantee the performance of our particle technology for specific applications. Nanotrap® particles conform to physical and performance criteria for 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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The Nanotrap® particles are the subject of numerous United States and foreign patent applications.
Ceres will not be responsible for violations or patent infringements that may occur with the use of our products.

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