NEAT Liquid Biopsy Kit User Guide

Manual cfDNA Isolation with 4 mL of Plasma Collected in a Streck Cell-Free DNA BCT

This protocol is optimized to effectively capture and concentrate cfDNA while excluding genomic DNA from 4 mL of plasma collected in Streck Cell-Free DNA BCT® for use with a user-friendly, magnetic particle-based manual method.

Kit Contents and Equipment Lists

Download the Kit Contents and Equipment Lists PDF at https://www.ceresnano.com/user-guide-neat.

Troubleshooting

- Ensure that all reagents are stored at the indicated temperatures.
- Make sure that refrigerated reagents and plasma samples (if applicable) are warmed to room temperature before starting the procedure.
- Nanotrap® Liquid Biopsy Particles and Nanotrap® Bind reagents must be adequately vortexed immediately before each use.
- Nanotrap® Elute must be pipetted carefully into the bottom center of each well.
  – Spin plate to bring content to bottom center if needed.
- Add the reagents in the order listed in the corresponding procedure.
  – Do not mix Nanotrap Liquid Biopsy Particles directly with Nanotrap® Proteinase K.
- Avoid using plasma samples that have undergone a freeze-thaw cycle for optimal sample integrity.

Kit Guidelines

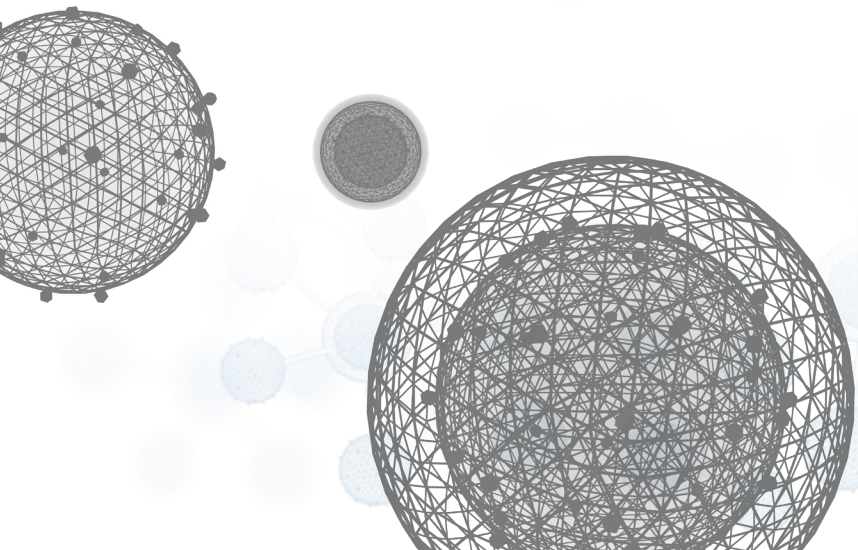
- **If using the NEAT Liquid Biopsy Kit for the first time:**
  – Resuspend Nanotrap Proteinase K with 12.5 mL sterile, molecular biology grade water.
- **Reagent Preparation:**
  – Prepare an 80% ethanol solution using 100% laboratory grade ethanol and molecular biology grade water.
- **Perform all steps in the protocols at room temperature (15°C to 30°C):**
  – Allow Nanotrap Liquid Biopsy Particles, Nanotrap Proteinase K, 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 Liquid Biopsy Particles and Nanotrap Bind must be vortexed for at least 30 seconds immediately before each use to ensure that the component is fully resuspended.**

Preparation of Plasma from Whole Blood

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.
3. Centrifuge the plasma at 16,000 x g for 10 minutes at 4°C.
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.



Experimental Setup

1. Preheat the thermal mixer to 60°C.

Nanotrap Particle Capture

In a 15 mL centrifuge tube, complete in order:

1. Add **800 µL** of **Nanotrap® Adapt** to the sample tube.
2. Invert plasma gently 5 times. Add **4000 µL** of **plasma** to the sample tube.
3. Vortex Nanotrap Liquid Biopsy Particles for 30 seconds to resuspend.
4. Add **1000 µL** of **Nanotrap Liquid Biopsy Particles** to the sample tube.
5. Add **400 µL** of **Nanotrap Proteinase K** to the sample tube.
6. Add **1300 µL** of **Nanotrap® Lyse** to the sample tube.
7. Vortex the sample tube for 30 seconds.
8. Incubate the sample in the thermal mixer at 60°C, at 750 rpm, for 45 minutes.
9. Remove the sample tube and place on the tube rotator for 15 minutes.

Wash

1. Place the tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and discard.
2. Add **1000 µL** of **Nanotrap® Wash** to the sample tube then vortex for 15 seconds.
3. Transfer the sample to a new 1.5 mL tube and save the 15 mL sample tube.
4. Place the 1.5 tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and rinse any remaining sample from the 15 mL sample tube with removed supernatant.
5. Transfer any remaining sample from 15 mL sample tube to the 1.5 mL tube.
6. Place the tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and discard.
7. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
8. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and discard.
9. Remove the remaining supernatant with a P-20 pipette and discard.

Transfer and Bind

1. Add **500 µL** of **Nanotrap® Transfer** to the sample tube and vortex for 15 seconds.
2. Place the sample tube on the tube rotator for 10 minutes.
3. Centrifuge sample tube for 5 seconds to bring contents to the bottom of the tube.
4. Place the tube on the magnet rack for 30 seconds or until the sample is clear.

5. **Transfer supernatant (now containing cfDNA) into a new 1.5 mL tube.** Ensure that all the supernatant is transferred. Discard the previously used tube containing the Nanotrap Liquid Biopsy Particles.
6. Vortex the Nanotrap Bind for 30 seconds to resuspend.
7. Add **30 µL** of **Nanotrap Bind** to the sample tube and vortex for 15 seconds.
8. Place the sample tube on the tube rotator for 15 minutes.
9. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
10. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove supernatant and discard.

Note: *The cfDNA is now bound to the Nanotrap Bind.*

Ethanol Wash

1. Add **200 µL** of **80% ethanol** to the sample tube and vortex for 15 seconds.
2. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove supernatant and discard.
3. Add **200 µL** of **80% ethanol** to the sample tube and vortex for 15 seconds.
4. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
5. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove supernatant and discard.
6. Tap the magnet rack (with sample tube) at least 5 times on the benchtop to bring the last of the supernatant to the bottom of the tube.
7. Remove the remaining supernatant with a P-20 pipette and discard.
8. Remove the tube from the magnet rack and open the cap to let the sample air dry for 3 minutes.

Elution

1. Add **20 µL** of **Nanotrap Elute** to the sample and vortex for 15 seconds.
2. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
3. Place the sample tube on a vortexer with an adaptor for 20 minutes on low.
4. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
5. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear.
6. Pipette the 20 µL eluant into a new, DNase-free tube. (Strip 0.2 mL PCR tubes are recommended.)

Samples are ready for analysis.

For optimal sample integrity, it is strongly advised to use them immediately. Store samples at 4°C for same-day use or at –80°C for long-term storage.

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