NEAT Liquid Biopsy Kit User Guide

Manual cfDNA Isolation with 1 mL of Plasma Collected in a K2EDTA Blood Collection Tube or a PAXgene Blood ccfDNA Tube

This protocol is optimized to capture and concentrate cfDNA while excluding genomic DNA from 1 mL of plasma collected in K2EDTA Blood Collection Tubes or PAXgene® Blood ccfDNA Tubes with a user-friendly, magnetic particle-based manual method.

Kit Contents and Equipment Lists

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

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

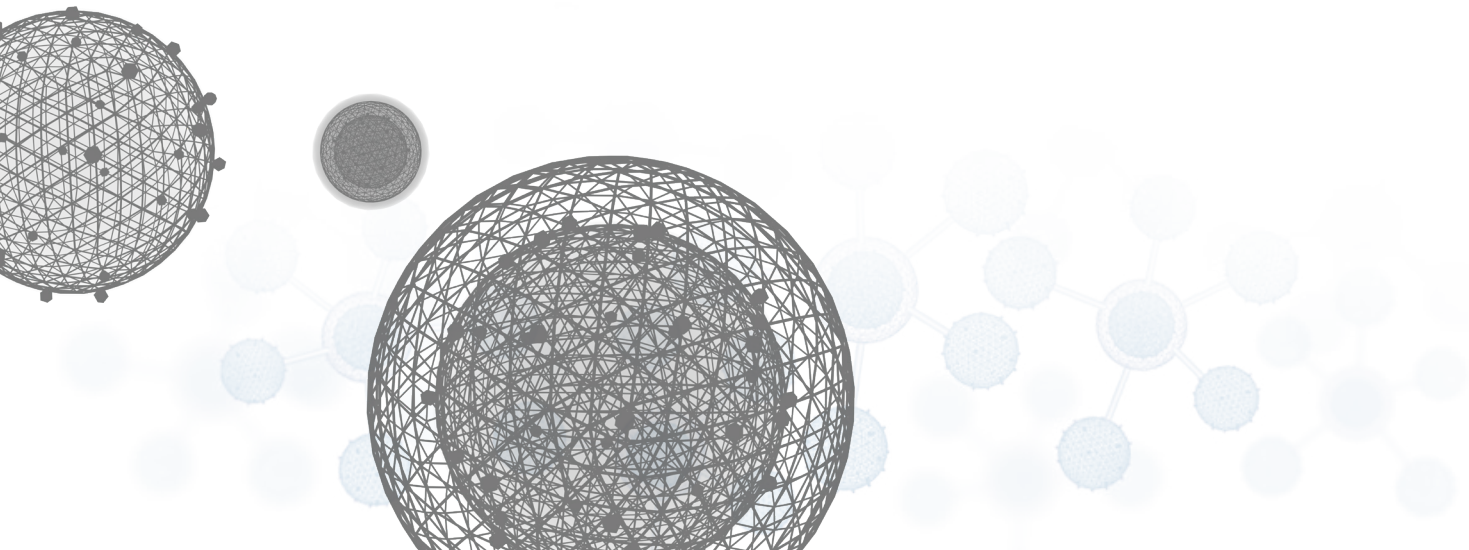
- **If using the NEAT Liquid Biopsy Kit for the first time:**
  – Resuspended Proteinase K should be aliquoted and stored for up to 12 months at −15 to −25°C.
  Avoid repeated freezing and thawing as this may lead to precipitation of the protein.
- **Reagent Preparation:**
  – Prepare an 80% ethanol 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

1. Centrifuge whole blood samples according to the blood collection tube manufacturer guidelines.
2. Carefully transfer plasma to a new tube (or directly to sample plates).

Only one spin is needed for use with the NEAT Liquid Biopsy Kit.

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



Experimental Setup

1. Preheat the thermal mixer to 45°C.

Nanotrap Particle Capture

In a 2 mL microcentrifuge tube, complete in order:

1. Vortex Nanotrap Liquid Biopsy Particles for 30 seconds to resuspend.
2. Invert plasma gently 5 times. Add **1000 µL** of **plasma** to the sample tube.
3. Add **250 µL** of **Nanotrap Liquid Biopsy Particles** to the sample tube.
4. Add **100 µL** of **Nanotrap Proteinase K** to the sample tube.
5. Add **600 µL** of **Nanotrap® Lyse** to the sample tube.
6. Vortex the sample tube for 30 seconds.
7. Incubate the sample in the thermal mixer at 45°C, at 750 rpm, for 20 minutes.

Wash

1. Centrifuge the sample tube for 5 seconds to bring the contents to the bottom of the tube.
2. Place the tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and discard.
3. Add **1000 µL** of **Nanotrap® Wash** 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 the 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.

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 the 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 **15 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

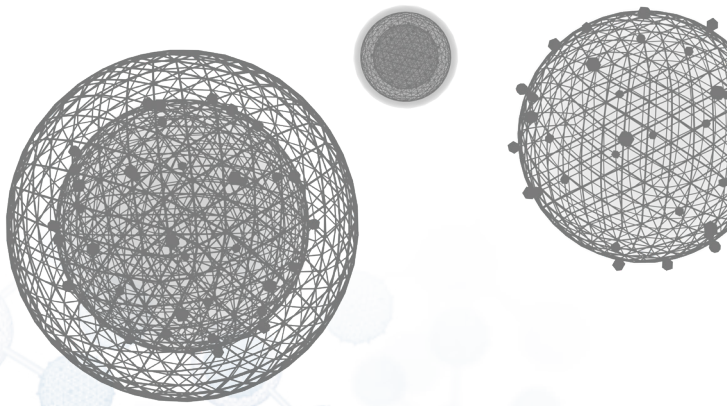
1. Add **200 µL** of **80% ethanol** to the sample tube and vortex for 15 seconds.
2. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove supernatant and discard.
3. Add **200 µL** of **80% ethanol** to the sample tube and vortex for 15 seconds.
4. Centrifuge 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 of cfDNA

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. Incubate the sample on the benchtop for 3 minutes for cfDNA elution.
4. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear.
5. 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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October 2024
Literature # PL-UG31359, APP-213