NEAT Liquid Biopsy Kit User Guide

Manual cfDNA Isolation with 4 mL of Plasma Collected in a K2EDTA Blood Collection Tube or a PAXgene Blood ccfDNA Tube

This protocol is optimized to effectively capture and concentrate cell-free DNA while excluding genomic DNA from 4 mL of plasma collected in K2EDTA Blood Collection Tubes or PAXgene Blood ccfDNA Tubes with a user-friendly, magnetic particle-based manual method.

Kit Guidelines

- **If using the Nanotrap® 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 anything that has settled during storage is fully resuspended.**

Reagents and Materials Not Included

- Ethanol (200 Proof)
- Molecular Biology Grade Water, Sterile
- Pipette tips
- 1.5 mL, 2 mL, and 15 mL centrifuge tubes – Lo-bind tubes recommended
- 0.2 mL PCR strip tubes (DNase/RNase Free) – not required, recommended

Equipment

- Benchtop Centrifuge (capable of 1,600 x g) for collecting plasma from whole blood
- High-speed centrifuge (capable of 16,000 x g) for collecting plasma from whole blood
- Single-channel pipette(s)
- Magnetic Racks for **2 mL** and **15 mL** tube sizes - Thermo Fisher DynaMag™ racks recommended
- Laboratory thermal mixer – Eppendorf® Thermomixer® or Benchmark MultiTherm Shaker Touch recommended

- Mini-Centrifuge
- Vortexer
- Tube rotator – Benchmark ROTOMINI PLUS recommended

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, they can be kept frozen at ≤ - 80°C for long-term storage.

Experimental Setup

1. Preheat the thermal mixer to 45°C.

Nanotrap® Particle Capture

In a 15 mL centrifuge tube, complete in order:

1. Invert plasma gently 5 times. Add **4000 µL** of **plasma** to the sample tube.
2. Vortex **Nanotrap Liquid Biopsy Particles** for 30 seconds to resuspend.
3. Add **1000 µL** of **Nanotrap Liquid Biopsy Particles** to the sample tube.
4. Add **200 µL** of **Nanotrap Proteinase K** to the sample tube.
5. Add **1300 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, 750 rpm for 20 minutes.

Wash

1. Place the tube on the magnet rack for 30 seconds, or until the sample is clear. Then, remove the supernatant and discard it.
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 the removed supernatant.
5. Transfer any remaining sample from the 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. Then, remove the supernatant and discard it.
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 the sample tube for 5 seconds to bring the 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 the supernatant** (now containing cfDNA) into a new 1.5 mL tube, ensuring 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

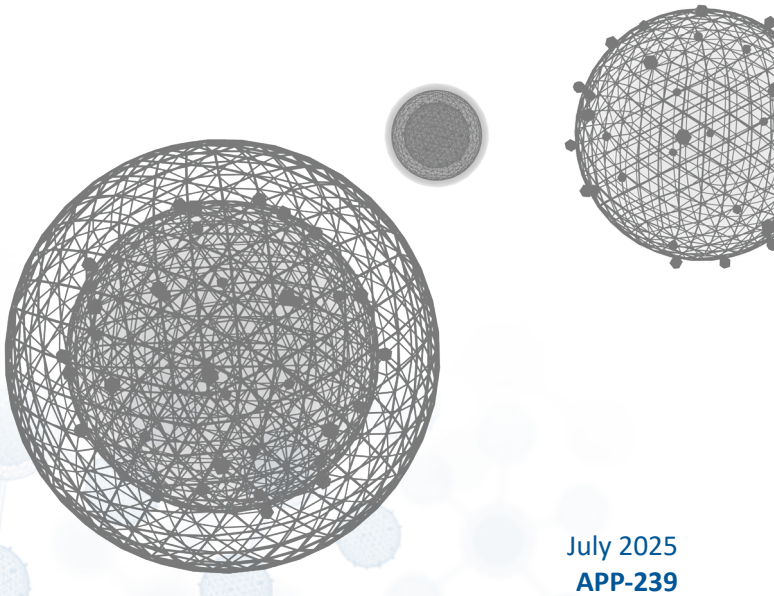
1. Add **200 µL** of **80% Ethanol** to the sample tube and vortex for 15 seconds.
2. Place the sample tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and discard.
3. Add **200 µL** of **80% Ethanol** to the sample tube and vortex for 15 seconds.
4. Centrifuge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

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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