NEAT Liquid Biopsy Kit User Guide

Automated cfDNA Isolation on KingFisher Apex/Flex System 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 cell-free DNA (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 method for running up to 96 samples at once. This protocol utilizes a KingFisher™ Apex System or a KingFisher™ Flex System for sample processing.

Kit Contents and Equipment Lists

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

Automated Scripts for KingFisher Apex and Flex Systems

For KingFisher scripts, contact sales@ceresnano.com.

1 mL *[K2EDTA Tube or PAXgene ccfDNA Tube Derived Plasma]*

– **KingFisher Apex:** 1mL_NEATLB_APEX_V1
– **KingFisher Flex:** 1mL_NEATLB_FLEX_V1

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.
- Do not allow the Nanotrap Bind to dry out in the “Bind” plate prior to adding the Transfer solutions and ensure that it is properly pipetted into the bottom center of the wells.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.

- **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. 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.

Procedure Setup

1. Collect and label (7) KingFisher™ 96 Deep-well Plates, as follows:
   "Lyse 1," "Lyse 2," "Wash," "Bind," "Ethanol Wash 1," "Ethanol Wash 2".
2. Collect and label (1) KingFisher™ 96 Plate (200 µL), as follows: "Elute".
3. Collect (2) KingFisher™ 96 Deep-well Tip Combs.

Prepare Lyse Plates

Important Note: A plasma sample is divided between the same wells of the "Lyse 1" and "Lyse 2" plates. For example, place half the input volume of plasma sample A into well A1 of the "Lyse 1" plate and the remaining input volume of plasma sample A into well A1 of the "Lyse 2" plate. Next, repeat with plasma sample B into well A2, and so on.

"Lyse 1" and "Lyse 2" plates are identical in layout and contents. FILL IN ORDER:

1. Invert the plasma tube(s) gently 5 times.
2. Add **500 µL** of **plasma** into the "Lyse 1" 96 deep-well plate, then add **500 µL** of the same **plasma** sample into the corresponding well placement in the "Lyse 2" 96 deep-well plate.
3. Vortex Nanotrap Liquid Biopsy Particles for 30 seconds to resuspend.
4. Add **125 µL** of **Nanotrap Liquid Biopsy Particles** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."
5. Add **25 µL** of **Nanotrap Proteinase K** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."
6. Add **250 µL** of **Nanotrap® Lyse** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."

Prepare Wash, Transfer, Ethanol Wash, and Elution Plates

Important Note: Add reagents to the specified plates in the corresponding wells that contain plasma samples. For example, if there are plasma samples in wells A1–A3 in the "Lyse 1" plate, add reagent to wells A1–A3 in the subsequent plates.

1. Add **1000 µL** of **Nanotrap® Wash** to the "Wash" 96 deep-well plate for each plasma sample.
2. Add **500 µL** of **Nanotrap® Transfer** to the "Transfer" 96 deep-well plate for each plasma sample.
3. Add **250 µL** of **80% Ethanol** to the "Ethanol Wash 1" 96 deep-well plate for each plasma sample.
4. Add **60 µL** of **80% Ethanol** to the "Ethanol Wash 2" 96 deep-well plate for each plasma sample.
5. Add **25 µL** of **Nanotrap Elute** to the "Elute" 96 plate (200 µL) for each plasma sample, carefully pipetting directly to the bottom center of the well.

Prepare Bind Plate

Important Note: Add reagents to the specified plates in the corresponding wells that contain plasma samples. For example, if there are plasma samples in wells A1–A3 in the "Lyse 1" plate, add reagent to wells A1–A3 in the subsequent plates.

1. Add **25 µL** of **Molecular Biology Grade Water** to the "Bind" 96 deep-well plate for each plasma sample.
2. Vortex the **Nanotrap Bind** for 30 seconds to resuspend.
3. Add **25 µL** of **Nanotrap Bind** to the "Bind" plate for each plasma sample.
4. Place (1) 96 deep-well tip comb into the "Bind" plate.

KingFisher Protocol Run

1. Place tip combs in "Lyse 1" and "Bind" plates for the 1 mL protocol.
2. Download and start the KingFisher script and follow the on-screen instructions to load the prepared "Lyse 1," "Lyse 2," "Wash," "Bind," "Ethanol Wash 1," "Ethanol Wash 2," and "Elute" plates into the instrument and run the protocol.
   - **KingFisher APEX Script: 1mL_NEATLB_APEX_V1.kfx**
   - **KingFisher FLEX Script: 1mL_NEATLB_FLEX_V1.bdz**
3. At the completion of the KingFisher run, remove the plates from the KingFisher System as prompted. Cover the "Elute" plate with parafilm or a plate sealing film and save until ready for use. This plate contains the concentrated cfDNA.

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.

QUICK GUIDE: Automated 1 mL of Plasma Collected in a K2 EDTA Blood Collection Tube or a PAXgene Blood ccfDNA Tube

NEAT Liquid Biopsy Kit Quick Guide 1 mL KingFisher Automated cfDNA isolation	
KingFisher Plate	**Reagents Added**
Lyse 1 96 Deep-well Plate + Tip Comb	**500 µL** plasma from K2 EDTA Blood Collection Tubes or PAXgene Blood ccfDNA Tubes
	125 µL NEAT Liquid Biopsy Particles
	25 µL Nanotrap Proteinase K
	250 µL Nanotrap Lyse
Lyse 2 96 Deep-well Plate	**500 µL** plasma from K2 EDTA Blood Collection Tubes or PAXgene Blood ccfDNA Tubes
	125 µL NEAT Liquid Biopsy Particles
	25 µL Nanotrap Proteinase K
	250 µL Nanotrap Lyse
Wash 96 Deep-well Plate	**1000 µL** Nanotrap Wash
Transfer 96 Deep-well Plate	**500 µL** Nanotrap Transfer
Bind 96 Deep-well Plate + Tip Comb	**25 µL** Molecular Biology Grade Water
	25 µL Nanotrap Bind
Ethanol Wash 1 96 Deep-well Plate	**250 µL** 80% Ethanol
Ethanol Wash 2 96 Deep-well Plate	**60 µL** 80% Ethanol
Elute 96 Plate (200µL)	**20 µL** Nanotrap Elute
Run KingFisher Script: 1mL_NEATLB_APEX_V1 **or** 1mL_NEATLB_FLEX_V1	

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January 2024
Literature # PL-UG31354