NEAT Liquid Biopsy Kit User Guide

Automated cfDNA Isolation on KingFisher Apex/Flex System with 4 mL of Plasma Collected in a Streck Cell-Free DNA BCT

This protocol is optimized to capture and concentrate cfDNA while excluding genomic DNA from 4 mL of plasma collected in a Streck Cell-Free DNA BCT® for use with a user-friendly method for running up to 24 samples at once. This protocol utilizes a KingFisher™ Apex System or a KingFisher™ Flex System for sample processing.

This protocol involves two serial runs on the KingFisher System. The first run is in a 24-well format, and at the end the cfDNA is in Nanotrap® Transfer. The Nanotrap Transfer solution is moved to a new 96-well plate for the second run of the KingFisher System protocol. Up to four plates from the first run can be combined and run together in the 96-well format for the second run. At the end of the second run, the captured and concentrated cfDNA is in Nanotrap® Elute and ready for downstream analysis.

Kit Contents and Equipment Lists

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

Automated Scripts for KingFisher Apex and Flex

For KingFisher scripts, contact sales@ceresnano.com.

4 mL *[Streck Cell-Free DNA BCT Plasma]*

– **KingFisher Apex:** 24_4mL_NEATLB_STRECK_APEX_V1 and 96_4mL_NEATLB_STRECK_APEX_V1
– **KingFisher Flex:** 24_4mL_NEATLB_STRECK_V1 and 96_4mL_NEATLB_STRECK_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.
- Seal the ethanol wash plate or elution plates during the first run to avoid reagent evaporation and unseal just before loading the second run for protocols that involve two KingFisher System runs.
- Do not allow the Nanotrap Bind to dry out in the “Bind” plate prior to adding the Nanotrap 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.

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

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 plates).

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

First Run

Procedure Setup

1. Collect and label (5) KingFisher™ 24 Deep-well Plates, as follows: "Tip Comb 1," "Lyse 1," "Lyse 2," "Wash," and "Transfer."
2. Collect and place a KingFisher™ 24 Deep-well Tip Comb into the "Tip Comb 1" plate and set aside for *KingFisher Protocol, First Run*.

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. Add **400 µL** of **Nanotrap® Adapt** to each well that will contain a plasma sample in the "Lyse 1" 24 deep-well plate and "Lyse 2" 24 deep-well plate.
2. Invert the plasma tube(s) gently 5 times.
3. Add **2000 µL** of **plasma** into the "Lyse 1" plate, then add **2000 µL** of the same **plasma** sample into the corresponding well placement in the "Lyse 2" plate.
4. Vortex Nanotrap Liquid Biopsy Particles for 30 seconds to resuspend.
5. Add **500 µL** of **Nanotrap Liquid Biopsy Particles** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."
6. Add **200 µL** of **Nanotrap Proteinase K** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."
7. Add **650 µL** of **Nanotrap® Lyse** to each well containing a plasma sample in plates "Lyse 1" and "Lyse 2."

Prepare Wash and Transfer 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" and "Lyse 2" plates, add reagent to wells A1–A3 in the subsequent plates.

1. Add **1000 µL** of **Nanotrap® Wash** to the "Wash" 24 deep-well plate for each plasma sample.
2. Add **500 µL** of **Nanotrap Transfer** to the "Transfer" 24 deep-well plate for each plasma sample.

KingFisher Protocol, First Run

1. Download and start the KingFisher script and follow the on-screen instructions to load the prepared "Tip Comb 1," "Lyse 1," "Lyse 2," "Wash," and "Transfer" plates into the instrument and run the protocol.
   - **KingFisher APEX Script: 24_4mL_NEATLB_STRECK _APEX_V1.kfx**
   - **KingFisher FLEX Script: 24_4mL_NEATLB_STRECK _V1.bdz**
2. At the completion of the first KingFisher run, remove the plates from the KingFisher system as prompted. ***Do not discard the "Transfer" Plate.*** The "Transfer" Plate now contains the cfDNA that was captured from the plasma samples and will be used in the *KingFisher Protocol, Second Run*. cfDNA in Nanotrap Transfer may be sealed and stored for up to one hour at room temperature.

Second Run

Procedure Setup

1. Collect and label (4) KingFisher™ 96 Deep-well Plates, as follows: “Tip Comb 2,” “Bind,” “Ethanol Wash 1,” “Ethanol Wash 2.”
2. Collect and label (1) KingFisher™ 96 Plate (200 µL), as follows: “Elute.”
3. Collect and place a KingFisher™ 96 Deep-well Tip Comb into the “Tip Comb 2” Plate and set aside for *KingFisher Protocol, Second Run*.

Prepare Ethanol Wash and Elution Plates

Important Note: Add reagents to the specified plates in the corresponding wells for the number of plasma samples used.

1. Add **250 µL** of **80% ethanol** to the “Ethanol Wash 1” 96 deep-well plate for each sample.
2. Add **60 µL** of **80% ethanol** to the “Ethanol Wash 2” 96 deep-well plate for each sample.
3. Add **20 µL** of **Nanotrap Elute** to the “Elute” 96 plate (200 µL) for each sample, carefully pipetting directly to the bottom center of the well.
4. Cover the “Ethanol Wash 1,” “Ethanol Wash 2,” and “Elute” plates with parafilm or a plate sealing film and set aside for *KingFisher Protocol, Second Run*.

Note: *These plates may be prepped after the KingFisher Protocol, First Run, at user discretion.*

Prepare Bind Plate

1. Vortex the Nanotrap Bind for 30 seconds to resuspend.
2. Add **30 µL** of **Nanotrap Bind** to the “Bind” plate for each plasma sample used to the corresponding well placement in the “Ethanol Wash 1,” “Ethanol Wash 2,” and “Elute” plates.
3. **Pipette all the Nanotrap Transfer** from each well of the “Transfer” plate, taken from the KingFisher System after the first run, to the “Bind” plate to the corresponding wells that now contain Nanotrap Bind.

KingFisher Protocol, Second Run

1. Collect the filled “Ethanol Wash 1,” “Ethanol Wash 2,” and “Elute” plates and remove the plate covers.
2. Download and start the KingFisher script and follow the on-screen instructions to load the prepared “Tip Comb 2,” “Bind,” “Ethanol Wash 1,” “Ethanol Wash 2,” and “Elute” plates into the instrument and run the protocol.
   - **KingFisher APEX Script: 96_4mL_NEATLB_STRECK _APEX_V1.kfx**
   - **KingFisher FLEX Script: 96_4mL_NEATLB_STRECK _V1.bdz**
3. After the second KingFisher run is completed, remove the plates from the KingFisher System as prompted.
4. 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 4 mL of Plasma Collected in a Streck Cell-Free DNA BCT

NEAT Liquid Biopsy Kit – Streck BCT Quick Guide 4 mL Streck Cell-Free DNA BCT KingFisher Automated cfDNA Isolation	
KingFisher Plate	**Reagents Added**
Tip Comb 1 24 Deep-well Plate + Tip Comb	**NONE**
Lyse 1 24 Deep-well Plate	**400 µL** Nanotrap Adapt
	2000 µL plasma from Streck Cell-Free DNA BCT
	500 µL NEAT Liquid Biopsy Particles
	200 µL Nanotrap Proteinase K
	650 µL Nanotrap Lyse
Lyse 2 24 Deep-well Plate	**400 µL** Nanotrap Adapt
	2000 µL plasma from Streck Cell-Free DNA BCT
	500 µL NEAT Liquid Biopsy Particles
	200 µL Nanotrap Proteinase K
	650 µL Nanotrap Lyse
Wash 24 Deep-well Plate	**1000 µL** Nanotrap Wash
Transfer 24 Deep-well Plate	**500 µL** Nanotrap Transfer
Run NEAT Liquid Biopsy Kit - Streck BCT Script: ***24_4mL_NEATLB_STRECK_APEX_V1*** **or** ***24_4mL_NEATLB_STRECK _V1*** ***Manual Transfer of Nanotrap Transfer Solution to Bind Plate***	
Tip Comb 2 96 Deep-well Plate + Tip Comb	**NONE**
Bind 96 Deep-well Plate	**30 µL** Nanotrap Bind
	500 µL Transfer Plate Solution *(manually transferred)*
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: ***96_4mL_NEATLB_STRECK _APEX_V1*** **or** ***96_4mL_NEATLB_STRECK _V1***	

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