Nanotrap® Extraction Advanced Technology (NEAT) Liquid Biopsy Kit - Streck BCT

INSTRUCTIONS FOR USE

INTENDED USE

Ceres Nanotrap® Extraction Advanced Technology (NEAT) Liquid Biopsy Kit - Streck BCT is intended for use in capturing and concentrating cell-free DNA (cfDNA) while excluding genomic DNA from double-spun plasma collected in Streck Cell-Free DNA BCT® or Nucleic Acid BCT™.

SUMMARY AND PRINCIPLES

The NEAT Liquid Biopsy Kit - Streck BCT uses the Nanotrap hydrogel particle technology to capture and concentrate cfDNA from plasma samples while simultaneously reducing genomic DNA contamination. The result is a purer and more concentrated cfDNA product, 50-800 base pair fragments, that can be used in downstream qPCR, dPCR, ddPCR, and/or sequencing assays.

PRECAUTIONS

1. **For Research Use Only. Not for use in diagnostic procedures.**
2. SDS for kit reagents can be obtained at streck.com, by calling 800-843-0912 or by calling your local supplier.
3. Ensure that all reagents are stored at the indicated temperatures.
4. Refrigerated reagents and plasma samples should be warmed to room temperature before starting the procedure.
5. Nanotrap Liquid Biopsy Particles and Nanotrap Bind Reagents must be adequately vortexed immediately before each use.
6. Nanotrap Elutes must be pipetted carefully into the bottom center of each well. Spin plate to bring contents to bottom of center if needed.
7. Add reagents in the order listed in the procedure. DO NOT mix Nanotrap Liquid Biopsy Particles directly with Nanotrap Proteinase K. Direct mixing will render the particles unusable.
8. Avoid using plasma samples that have undergone a freeze-thaw cycle for optimal sample integrity.
9. Do not use bleach with this product.

STORAGE AND STABILITY

1. The large kit box containing Nanotrap Lyse, Wash, Elute, Transfer, and Adapt should be stored at 15 °C to 30 °C. The smaller kit box containing Nanotrap Liquid Biopsy Particles, Proteinase K, and Bind should be stored at 2 °C to 8 °C. **DO NOT FREEZE**.
2. When stored at the proper temperature range, product contents are stable through the expiration date.
3. The Nanotrap Liquid Biopsy Particles are light sensitive and should be protected from light.

INDICATIONS OF PRODUCT DETERIORATION

1. Precipitate may form if product has previously been frozen. If aggregates are present, product should not be used.

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Kit Guidelines

1. If using the kit for the first time, resuspend Nanotrap Proteinase K with 12.5ml sterile, molecular biology grade water.
2. Once resuspended, Proteinase K should be aliquoted and stored for up to 12 months at -15 °C to -25 °C. Avoid repeated freezing and thawing, as this may lead to precipitation of the protein.
3. Prepare an 80% ethanol solution using 100% laboratory grade ethanol and molecular biology water.
4. Perform all steps at room temperature (15 °C to 30 °C) by allowing Nanotrap liquid biopsy particles, Nanotrap Proteinase K, and plasma to reach room temperature. If plasma is frozen, it may be placed at 37 °C until fully thawed.
5. 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.
6. This kit is compatible with 1-4ml plasma volume input. The following protocol is designed for 4ml of plasma. Adjust reagent ratios if using a smaller volume.

Plasma Preparation from Whole Blood

1. Centrifuge whole blood samples at 1600 x g for 10 minutes at room temperature.
2. Carefully transfer plasma to a new tube.
3. Centrifuge plasma at 16,000 x g for 10 minutes at 4 °C.
4. Carefully transfer plasma into a new tube or directly into sample tubes.
   Note: after transferring plasma samples into a new tube, plasma may be stored at 2 °C to 8 °C for up to 24 hours or at -80 °C +/- 10 °C for up to 45 days.

Nanotrap Particle Capture using Manual Method

Preheat thermal mixer to 60 °C.
In a 15ml centrifuge tube complete steps in the following order:

1. Add 800µl of Nanotrap Adapt to the sample tube.
2. Invert plasma gently 5 times. Add 4000µl of plasma to the sample tube.
3. Vortex Nanotrap Liquid Biopsy Particles for 30 seconds to resuspend.
4. Add 1000µl of Nanotrap Liquid Biopsy Particles to the sample tube.
5. Add 400µl of Nanotrap Proteinase K to the sample tube.
6. Add 1300µl 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 1000µl of Nanotrap Wash to the sample tube then vortex for 15 seconds.
3. Transfer the sample to a new 1.5ml tube and save the 15ml sample tube.
4. Place the 1.5ml tube on the magnet rack for 30 seconds or until the sample is clear. Remove the supernatant and rinse any remaining sample from the 15ml sample tube with removed supernatant.
5. Transfer any remaining sample from 15ml sample tube to the 1.5ml 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 500µl 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 1.5ml 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 30µl 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 200µl 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 200µl 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 sample 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 without replacing the cap to let the sample air dry for 3 minutes.

Elution

1. Add 20µl 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 20µl eluant into a new, DNase-free tube. (Strip 0.2ml PCR tubes are recommended.)
7. 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.

Automated protocols are platform specific. Automated protocols can be found at www.streck.com/protocols/.

Recommendations on materials to use with this kit can be found in the NEAT User Guide Equipment List.

ORDERING INFORMATION

Please call our Customer Service Department at 800-228-6090 for assistance. Additional information can be found online at streck.com.

TECHNICAL SUPPORT

Please contact Streck Technical Services at +1-402-691-7510 (outside the United States and Canada), 800-843-0912 (United States and Canada) or technicalservices@streck.com.

GLOSSARY OF SYMBOLS

See the Instructions (IFU) tab under Resources on the product page at streck.com.

See streck.com/patents for patents that may be applicable to this product.

Streck
7002 S. 109 Street
La Vista, NE 68128 USA

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