truXTRAC® FFPE
total NA Plus Kit – Magnetic Beads

Adaptive Focused Acoustics® (AFA)-based sequential RNA and DNA extraction from FFPE tissues using magnetic bead-based purification

PN 520255

INTENDED USE

The truXTRAC® FFPE total NA (Nucleic Acid) Plus Kit is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of any disease.

INTRODUCTION

The truXTRAC® FFPE total NA Plus Kit is designed for efficient and sequential extraction of total nucleic acids (RNA and DNA) from Formalin Fixed, Paraffin Embedded (FFPE) tissue samples using Covaris Adaptive Focused Acoustics (AFA®).

AFA-energetics® enables the active removal of paraffin from FFPE tissue samples in an aqueous buffer, while simultaneously rehydrating the tissue. Compared to traditional passive, chemical-based methods of paraffin removal, this non-contact mechanical process is more efficient as the paraffin is removed and emulsified from the tissue. Uniquely, AFA enables increased yields of nucleic acids and minimizes the degradation of nucleic acids exposed at the FFPE section surface. The truXTRAC protocol results in high yields of high-quality RNA and DNA for sensitive analytical methods such as next-generation sequencing (NGS) or qPCR/RT-qPCR.

This protocol is optimized for up to 30 µm of total FFPE tissue sections or two FFPE cores (1.2 mm in diameter and 5 mm in length). For samples of smaller input sizes, the truXTRAC total NA Kit (PN 520246) may be used for extraction and purification of DNA and RNA from FFPE samples.

Important Notes on FFPE Samples:

The yield of DNA and RNA from FFPE tissue blocks is highly variable. Factors such as fixation time, size and thickness of the cores, the ratio of tissue to paraffin, the type of tissue, and the age and storage conditions of the FFPE block are the main causes for this variability.

More importantly, however, the quality of DNA and RNA isolated from FFPE samples can also be highly variable. During the fixation process, DNA and RNA are cross-linked to proteins and other nucleic acid molecules to varying degrees. The nucleic acid fragment or strand length isolated from FFPE samples is generally shorter as compared to nucleic acids that are isolated from fresh or frozen tissues [1]. This is particularly evident in older FFPE sample blocks or sample blocks stored at elevated temperatures. Thus, an advanced mechanical deparaffinization process is important to extract the high quality nucleic acids required for sensitive analytical techniques. Covaris AFA enables non-contact mechanical removal of paraffin from FFPE samples to improve the yield and quality of extracted nucleic acids.

If you require any assistance with this product please refer to Troubleshooting (Appendix B) in this protocol, check the FAQs found on our website, or contact Covaris Application Support at ApplicationSupport@covaris.com.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010451	A	6/18	Initial release of truXTRAC FFPE total NA Plus Kit - Magnetic Beads
010451	B	7/18	Update protocol with new clarifications

KIT CONTENTS

○ NA plus Tissue Lysis Buffer	25 ml
○ Proteinase K (PK Solution)	3.5 ml
○ Magnetic Bead Suspension	0.5 ml
○ Buffer BB3	75 ml
○ Buffer WB3	60 ml
○ Buffer WB4	60 ml
○ RNA Elution Buffer	3.5 ml
○ Buffer BE	7.5 ml
○ microTUBE-500 AFA Fiber Screw-Cap FFPE	25

SDS INFORMATION IS AVAILABLE AT http://covaris.com/resources/safety-data-sheets/

STORAGE

Upon kit arrival, store the Proteinase K solution and the Magnetic Bead Suspension at 2-8C.

Store all other kit components at room temperature.

LABORATORY EQUIPMENT, CHEMICALS AND CONSUMABLES SUPPLIED BY USER

Required Laboratory Equipment and Accessories

- microTUBE-500 Centrifuge and Heat Block Adapter (Covaris, PN 500503)
- Dry block heater for 2 ml tubes or temperature-controlled water bath able to accurately heat between 50-90C
- Magnet Stand for 2 ml tubes (e.g. Thermo Fisher Scientific, DynaMag™-2 Magnet, PN 12321D)

Required Chemicals and Enzymes

- 100% ethanol, molecular biology grade (e.g., AmericanBio, PN AB00515)
- Nuclease-free water (e.g., Invitrogen, PN AM9930)

Optional Enzymes

- DNase TURBO DNA-free kit (Thermo Fisher Scientific, PN AM1907)
- Optional DNase-free RNase A (10 mg/ml) (e.g., Thermo Fisher Scientific, PN EN0531)

Required Consumables

- 2 ml nuclease free microfuge tubes (e.g., Eppendorf Safe-Lock Tubes, PN 022363352)

Optional Supplies for FFPE Sample Collection from Slides

- FFPE tissuePICK™ (Covaris, PN 520163)
- tissuePICK Forceps (5) (Covaris, PN 520164)
- FFPE sectionPICK™ (Covaris, PN 520149)
- FFPE sectionWARMER™ (Covaris, PN 500403)

Covaris Focused-ultrasonicator Accessories and Plate Definitions

The table below contains the parts and plate definitions necessary to run the protocol.

Use the parts and plate definitions specific to your Covaris Focused-ultrasonicator.

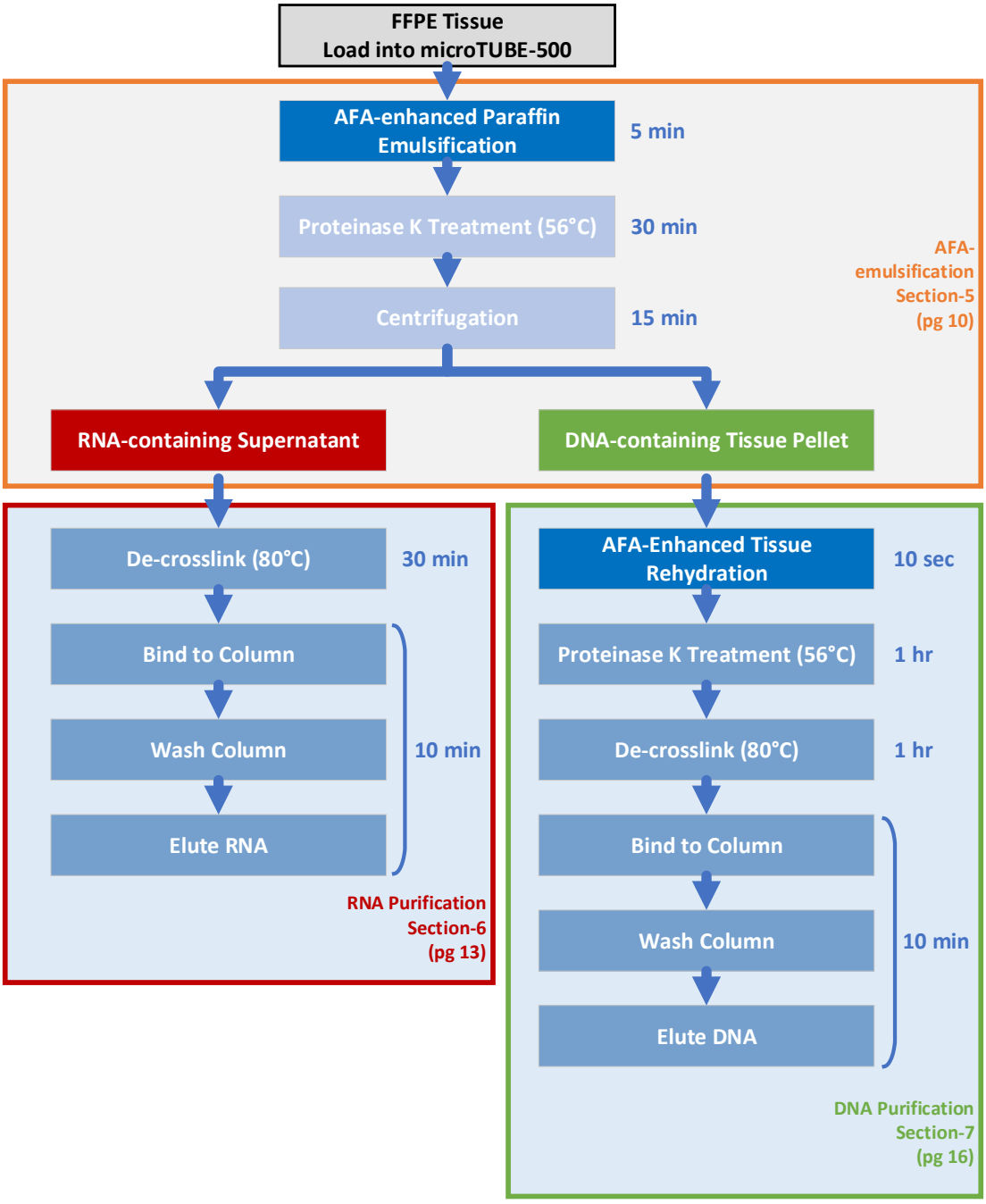
Instrument	M220	ME220	S220	E220 Evolution	E220	LE220 / LE220-plus
Holder/Rack Description (PN)	Holder XTU (500414)	Rack 4 Position microTUBE-500 (500525)	Holder microTUBE-500 Screw Cap (500449)	Rack E220e 4 microTUBE-500 Screw Cap (500484)	Rack 24 microTUBE-500 Screw Cap (500452)	Rack 24 microTUBE-500 Screw Cap (500452)
Plate definition file name	NA	<4 microTUBE-500 Screw-Cap PN 520185>	NA	<500484 E220e 4 microTUBE-500 Screw-Cap>	<500452 Rack 24 Place microTUBE-500 Screw-Cap>	<500452 Rack 24 Place microTUBE-500 Screw-Cap>
Required Accessories (PN)	Insert XTU (500471)	ME220 Waveguide 4 Place (500534)	NA	Intensifier (500141)	Intensifier (500141)	NA

FFPE tNA Extraction And Purification Workflow

Using the Adaptive Focused Acoustics (AFA) process, FFPE samples are prepared in NA Plus Tissue Lysis Buffer in the presence of Proteinase K, followed by an incubation at 56C for a short duration. This results in the release of RNA while minimizing over-digestion of the tissue and loss of genomic DNA.

The RNA-containing supernatant is separated from the DNA-containing tissue by a centrifugation step. RNA is then de-crosslinked and purified using magnetic beads.

Sequentially, DNA is released from the DNA-containing tissue by AFA-enhanced Proteinase K digestion, following a de-crosslinking step. DNA is then purified using magnetic beads.



1 – FFPE Sample Input Requirements and Guidelines

The truXTRAC protocol is highly efficient at mechanically removing paraffin, while simultaneously rehydrating the tissue.

> CAUTION: Do NOT exceed the input requirements in the tables below.
> Overloading will negatively impact the quality and quantity of extractable nucleic acids.

Slides section input requirements:

Scalpel or razor blade collection

Slide Collection Method	Maximum Input per microTUBE
Scalpel or razor blade to scrape material from slides	**30 µm of total thickness Area: 10 mm^2 (6 slides at 5 µm thick = 30 µm total thickness)**

FFPE tissuePICK™

Slide Collection Method	Maximum Input per microTUBE	Maximum FFPE Tissue Area per tissuePICK (5 µm thick section)
tissuePICK PN 520163	**2 tissuePICKs**	**100 mm^2**

FFPE sectionPICK™

Slide Collection Method	Maximum Input per microTUBE	Maximum FFPE Tissue Area per sectionPICK
sectionPICK PN 520149	**2 sections from a sectionPICK**	**Max Area: 100 mm^2 Thickness range: 4-30 µm**

> CAUTION: For optimal tissuePICK and sectionPICK performances, the tissue sections should be mounted on uncoated slides. tissuePICK and sectionPICK should always be used in conjunction with a Covaris sectionWARMER.

Curls/scrolls input requirements:

For best results, minimize the amount of wax present by trimming. We recommend no more than 1 part wax to 1 part tissue.

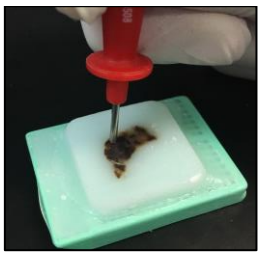
FFPE Curls/Scrolls Thickness	Maximum Scrolls per microTUBE
5 µm	**6**
10 µm	**3**
15 µm	**2**

FFPE core input requirements:

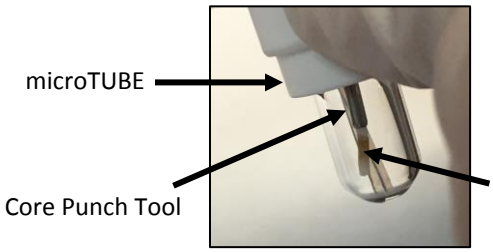
FFPE Core Punch Outer Diameter	Maximum Core Punches per microTUBE-500
≤ 1.2mm (15 Gauge, outer) Length = 5mm	**2**

Core punches may be loaded directly into the microTUBE-500 as shown below or transferred into the microTUBE-500 using tweezers or forceps.

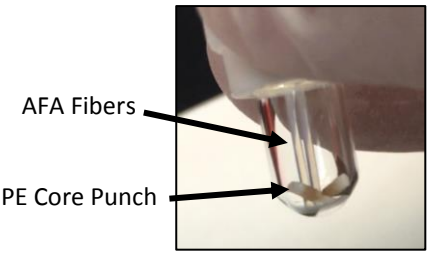
Core Punch Taken from Block



Loading Core Punch into microTUBE



Core Punches Loaded into microTUBE



2 – Preparation of Reagents

Follow these instructions before starting the FFPE tNA isolation protocol.

1. **NA Plus Tissue Lysis Buffer**: Check this buffer visually for a white precipitate that may form during storage. If white precipitate is visible, incubate the buffer bottles at 50 – 60C for 5 to 10 minutes before use to dissolve any precipitate.
2. **80% ethanol:** Prepare 80% ethanol by mixing 4 parts 100% ethanol with 1 part nuclease free water. One sample requires 2.3 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 2.6 ml.

3 – Preparation of Heat Blocks

1. Preheat dry block heaters to 56C and 80C ± 3C. It is critical that these temperatures are accurate in order to successfully execute the protocol.

2. Test the temperature of your water bath and heat blocks:
    a. Place a microcentrifuge tube (1.5 or 2 ml) filled with water into the heat block.
    b. Immerse a thermometer into the tube.
    c. Wait until the temperature has reached the plateau.
    d. Adjust the Set-temperature accordingly until the temperature inside the microcentrifuge tube has reached 56C or 80C ± 3C.

CAUTION: The Covaris microTUBE must be used in conjunction with Covaris Centrifuge and Heat Block microTUBE Adapters (PN 500503).

It is important to use an accurate heating source for incubation of microTUBE-500s and microcentrifuge tubes during Proteinase K and de-crosslinking incubations. Lower or higher than the indicated temperatures can adversely impact quality and quantity of purified nucleic acids.

4 – FOCUSED-ULTRASONICATOR SETUP

For detailed instructions on how to prepare and use your instrument, please refer to the respective Covaris User Manual. If you do not see a Plate Definition on your system, please contact Covaris Technical Support (techsupport@covaris.com)

Refer to page 4 for Plate Definitions and required Focused-ultrasonicator accessories

1. **Create "Acoustic Paraffin Emulsification" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "Acoustic Paraffin Emulsification" using the Covaris SonoLab method editor. Save the program for later use.

Instrument	M220	ME220	S220	E220 Evolution	E220	LE220
Peak Incident Power (PIP) (Watt)	75	75	200	200	200	450
Duty Factor (%)	25	20	10	10	10	20
Cycles Per Burst (CPB)	200	1000	200	200	200	200
Treatment time (seconds)	360	360	300	300	300	300
Bath temperature (°C)	20	20	20	20	20	20
Water Level (run)	Full	Auto	8	6	6	6

2. **Create "Acoustic Pellet Resuspension" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "Acoustic Pellet Resuspension" using the Covaris SonoLab method editor. Save the program for later use.

Instrument	M220	ME220	S220	E220 Evolution	E220	LE220
Peak Incident Power (PIP) (Watt)	75	75	200	200	200	450
Duty Factor (%)	25	20	30	30	30	30
Cycles Per Burst (CPB)	200	1000	200	200	200	200
Treatment time (seconds)	10	10	10	10	10	10
Bath temperature (°C)	20	20	20	20	20	20
Water Level (run)	Full	Auto	8	6	6	6

5 – Paraffin Emulsification, Tissue Rehydration, & Lysis

1. Prepare NA Plus Tissue Lysis Buffer/Proteinase K Mix by following instructions in Table 1 below and mix by inverting 10 times or vortexing for 3 seconds.

> ⚠ CAUTION: The NA Plus Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min after preparation.

Table 1 –Total NA Plus Tissue Lysis Buffer /Proteinase K Mix

Reagent	Volume for one sample*	Volume for N samples*
NA Plus Tissue Lysis Buffer	**440** µl	**440** µl x N
Proteinase K Solution	**44** µl	**44** µl x N

* calculation includes 10% excess in final volume

2. Open the microTUBE-500 Screw-Cap and load the FFPE tissue into the microTUBE-500 tube.

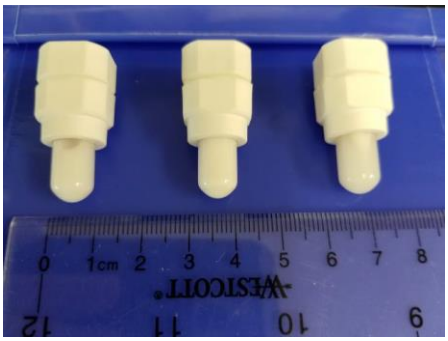
3. Add 440 µl NA Plus Tissue Lysis Buffer/Proteinase K mix. FFPE tissue may also be added directly to microTUBEs containing Lysis buffer. If adding FFPE tissue to microTUBEs containing Lysis buffer, ensure that the FFPE sample is fully immersed in the tube to prevent the sample from getting stuck in the Screw-Cap thread.

4. Close the microTUBE-500 tightly with the Screw-Cap and transfer the microTUBE-500 to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the microTUBE(s) into the Focused-ultrasonicator for processing.

5. Process the sample using the "***Acoustic Paraffin Emulsification***" program on the Focused-ultrasonicator.

   Note: It is expected that the solution will turn milky white. See example below.

Before AFA



After AFA

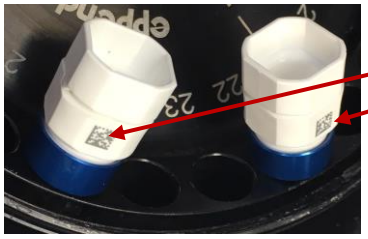
6. Remove the microTUBE-500 from the Focused-ultrasonicator and load it into the microTUBE-500 Centrifuge and Heat Block adapter.

When processing in batches, samples may be kept at room temperature for up to two hours prior to Proteinase K incubation at 56C (Step 7).

7. Incubate for 30 minutes at 56C. Remove microTUBE-500 together with the microTUBE-500 adapter from the heat block, and let cool at room temperature for 3 min.

> CAUTION: Do not chill on ice as rapid cooling will cause detergents to precipitate.

8. Place microTUBE-500 in the microTUBE-500 adapter with the bar code on the screw cap sleeve facing outward into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 15 minutes.



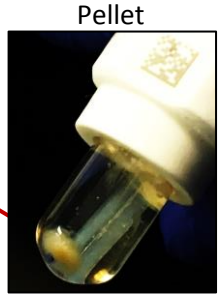
Note: The centrifuge lid may not close and may need to be left off during centrifugation.

9. Open the microTUBE and carefully transfer 400 µl supernatant into a 2 ml microcentrifuge tube.

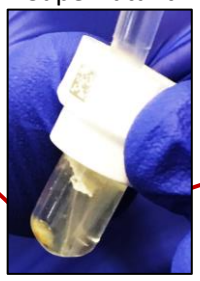
> CAUTION: This is a critical step in the workflow. By following these guidelines, the risk of losing the DNA-containing tissue pellet will be minimized:
>
> a. Locate the DNA-containing tissue pellet. It will be located on the same side as the barcode which faces outward during centrifugation. The pellet may appear faint and difficult to see.
> b. Tilt the tube slightly away from the pellet.
> c. Using a 200 µl pipette with a 200 µl pipette tip, slowly pierce the upper emulsified wax layer and carefully remove 200 µl of supernatant while simultaneously lowering the tip following the liquid level. Place the pipette tip towards the tube wall that faces away from the pellet and barcode. Use the same 200 µl pipette a second time to remove the remaining supernatant. DO NOT USE WIDE-MOUTH TIPS.
> d. A layer of emulsified wax may descend obscuring the pellet. This is normal. Leave 20-30 µl of supernatant behind. This will not significantly impact RNA or DNA yield.

Pre-centrifugation (No Pellet)

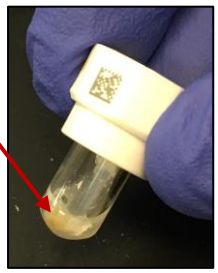
Post-Centrifugation Pellet



Removal of RNA Supernatant



Remaining Pellet



Note: If the pellet becomes dislodged from the wall of the microTUBE-500 before the RNA supernatant has been removed, repeat centrifugation (Step 8) to re-pellet the DNA. Remove RNA supernatant as described in Step 9.

10. Save the DNA-containing tissue pellet for subsequent DNA purification as described in Section-7. **Proceed immediately to RNA Purification (Section-6).**

    The DNA-containing pellet can be stored on ice or at 2–8C for up to 1 day. For longer periods, store at –15 to –30C.

6 - RNA PURIFICATION

1. Preheat dry block heaters to 56C (Steps 6 and 28) and 80C (Step 2) ± 3C as explained in Section-3.

2. Incubate the 2 ml microcentrifuge tube with the RNA-containing supernatant at 80C for 20 minutes. Remove tubes and cool at room temperature for 3 minutes.

3. Prepare BB3/ Magnetic Bead Mix according to Table 2 below.

Table 2 – BB3/Magnetic Bead Mix For RNA

Reagent	Volume for one sample*	Volume for N samples*
BB3	**1320**	**1320** µl x N
Magnetic Bead Suspension	**8.8**	**8.8** µl x N

*Calculation includes 10% excess

CAUTION: Vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using for 10 seconds to ensure a homogeneous suspension. Beads will settle when left standing.

4. Add 1208 µl of the BB3/Magnetic Bead Suspension mix to the RNA containing supernatant and cap the microcentrifuge tube.

5. Vortex the microcentrifuge tube for 10 seconds.

6. Incubate the microcentrifuge tube at 56C for 5 minutes.

7. Place the tube on a magnetic stand and incubate for 5 minutes until the beads have been pulled to the magnet.

CAUTION: With some samples, the binding supernatant may appear slightly brown after the 5 minute incubation on the magnet stand due to a small percentage of beads that do not migrate to the magnet. This effect does not reduce the yield significantly.

8. With the tube on the magnet, carefully remove the and discard the supernatant. Avoid touching or disturbing the bead pellet.

9. Remove the microcentrifuge tube from the magnetic stand and add 1 ml WB4.

10. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If beads are still sticking to the wall continue vortexing until all beads are resuspended.

11. Place the tube back on the magnetic stand and incubate for 5 minutes until the beads have been pulled to the magnet.

12. With the tube on the magnet, carefully remove and discard the supernatant.

Optional DNA removal step:

The truXTRAC FFPE total NA Plus Kit protocol isolates total RNA that may contain trace amounts of genomic DNA. If DNA-free RNA must be isolated, an optional DNase treatment can be performed.

Note: This optional DNase digestion must be performed after Step 12.

See Appendix A for step-by-step instructions.

13. Remove the tube from the magnetic stand and add 1 ml WB4.

14. Cap it and vortex thoroughly until all beads are resuspended.

15. Place the tube back on the magnetic stand and incubate for 5 minutes until the beads have been pulled to the magnet.

16. With the tube on the magnet, carefully remove and discard the supernatant.

17. After the final wash, remove as much of the supernatant as possible. Use a 20 µl pipettor to remove the remaining liquid from the bottom of the tube.

CAUTION: It is critical to remove the wash buffer supernatant completely because it contains residual paraffin. Remaining paraffin residue can result in bead clumping during elution and diminished yield.

18. Remove the tube from the magnetic stand and add 1 ml 80% ethanol.

19. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If beads are still sticking to the wall continue vortexing until all are resuspended.

20. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.

21. Remove and discard the supernatant without disturbing the bead pellet.

22. Remove the tube from the magnetic stand and add 300 µl 80% ethanol.

23. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended.

24. Place the tube on the magnetic stand and wait for 2 minutes until the beads have been pulled to the magnet.

25. Remove and discard as much of the supernatant as possible. Use a 20 µl pipet to remove the remaining liquid from the bottom of the tube.

26. Leave the tube open on the magnetic stand and let the beads dry for 6 minutes at room temperature.

CAUTION: Ensure that the ethanol has evaporated before continuing with elution. Residual ethanol can inhibit the elution and impact downstream applications such as PCR.

27. Remove the tube from the magnetic stand and add 50 to 100 µl of RNA Elution Buffer. Resuspend the beads by pipetting up and down 20 times. Ensure that all beads are resuspended in the buffer.

28. Cap the tube and incubate in the heat block set to 56C for 5 minutes.

29. Remove the tube from the heat block, place it on the magnetic stand, and incubate for 2 minutes.

30. Transfer the eluate into a clean elution tube without transferring beads. A small amount of residual paraffin may be visible in the pipet tip. This will not adversely affect downstream processing of the eluted RNA.

    Store the eluted RNA on ice until further processing.

    Isolated RNA should be kept at -80C for long term storage.

7 – DNA PURIFICATION

1. Preheat dry block heaters to 56C (Steps 7, 15 and 34) and 80C (Step 9) ± 3C. Place the heat block adapters in the heat block set to 56C.

2. Prepare NA Plus Tissue Lysis Buffer/Proteinase K Mix DNA in a microcentrifuge tube following instructions in Table 3 and mix by inverting 10 times or vortexing for 3 seconds.

CAUTION: The NA Plus Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min after preparation.

Table 3–NA Plus Tissue Lysis Buffer/Proteinase K Mix for DNA

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	**352** µl	**352** µl x N
Proteinase K Solution	**88** µl	**88** µl x N

* calculation includes 10% excess in final volume

3. Open the microTUBE with the DNA-containing tissue pellet and add 400 µl of the NA Plus Tissue Lysis Buffer/Proteinase K Mix for DNA.

4. Close the microTUBE-500 tightly with the Screw-Cap and transfer the microTUBE-500 to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the microTUBE(s) into the Focused-ultrasonicator for processing.

5. Process the sample using the "***Acoustic Pellet Resuspension***" program on your Covaris Focused-ultrasonicator.

6. Remove the microTUBE-500 from the Focused-ultrasonicator and load the microTUBE into the microTUBE-500 Centrifuge and Heat Block adapter on the heat block set to 56C.

7. Incubate for a minimum of 60 minutes at 56C.

   NOTE: The Proteinase K-treated sample can be stored at room temperature for up to an additional hour. Do not chill on ice.

CAUTION: It is recommended to increase the incubation time to 2 hours or up to overnight for core punch samples.

8. Remove microTUBE-500 together with the microTUBE-500 adapter from the heat block and transfer directly to dry heat block set-up for 80C incubation.

9. Incubate for 60 minutes at 80C.

10. Remove microTUBE-500 with adapter from the heat block and let cool for 3 minutes at room temperature.

11. Transfer the entire sample to a clean 2 ml microcentrifuge tube.

Optional RNA removal step:

At this point the sample can be treated with RNase A to remove residual RNA before continuing with DNA purification.

Add 5 µl of RNase A (10 mg/ml) solution and incubate for 5 minutes at room temperature, then continue to step 12.

12. Prepare BB3/Magnetic Bead Mix according to the Table 4.

Table 4 – BB3/Magnetic Bead Mix for DNA

Reagent	Volume for one sample	Volume for N samples*
BB3	**792** µl	**792** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

CAUTION: Vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using for 10 seconds to ensure a homogeneous suspension. Beads will settle when left standing.

13. Add 728 µl of the BB3/Magnetic Bead Mix to the DNA solution in the 2 ml microcentrifuge tube.

14. Cap the microcentrifuge tube and vortex for 10 seconds.

15. Incubate the microcentrifuge tube at 56C for 5 minutes.

16. Place the tube on a magnetic stand and wait for 5 minutes until the beads have been pulled to the magnet.

CAUTION: With some samples, the binding supernatant may appear slightly brown after the 5 minute incubation on the magnet stand due to a small percentage of beads that do not migrate to the magnet. This effect does not reduce the yield significantly.

17. With the tube still on the magnet, carefully remove and discard the supernatant. Avoid touching or disturbing the bead pellet.

18. Remove tube from the magnetic stand and add 1 ml Buffer WB3.

19. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If beads are still sticking to the wall continue vortexing until all are resuspended.

20. Place the tube back on the magnet stand and wait for 5 minutes until the beads have been pulled to the magnet.

21. With the tube on the magnet, carefully remove and discard the supernatant.

22. Repeat wash steps 18 through 21. After the final wash, remove as much of the supernatant as possible. Use a 20 µl pipettor to remove the remaining liquid from the bottom of the tube.

CAUTION: It is critical to remove the wash buffer supernatant completely because it contains residual paraffin. Remaining paraffin residue will result in bead clumping during elution and diminished yield.

23. Remove tube from the magnetic stand and add 1 ml 80% ethanol to the tube.

24. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If beads are still sticking to the wall continue vortexing until all are resuspended.

25. Place the tube on the magnetic stand and wait for 2 minutes until beads have been pulled to the magnet.

26. Remove the and discard supernatant without disturbing the bead pellet.

27. Remove the tube from the magnetic stand and add 300 µl 80% ethanol.

28. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended.

29. Place the tube on the magnetic stand and wait for 2 minutes until the beads have been pulled to the magnet.

30. Remove and discard as much of the supernatant as possible. Use a 20 µl pipet to remove the remaining liquid from the bottom of the tube.

31. Leave the tube open on the magnetic stand and let the beads dry for 6 minutes at room temperature.

CAUTION: Make sure that the ethanol has evaporated before continuing with elution. Residual ethanol can inhibit the elution and impact downstream applications such as PCR.

32. Remove the tube from the magnetic stand and add 50 to 100 µl of Buffer BE (5 mM TrisCl pH 8.5) into the tube.

33. Re-suspend the beads by pipetting up and down 20 times. Ensure that all beads are submerged in the buffer and are fully suspended.

34. Cap the tube and incubate the microcentrifuge tube in the heat block set to 56C for 5 minutes.

35. Remove the tube from the heat block and place it on the magnetic stand and wait for 2 minutes.

36. Transfer the eluate into a clean elution tube without transferring beads. A small amount of residual paraffin may be visible in the pipet tip. This will not adversely affect downstream processing of the eluted DNA.

Isolated DNA should be kept at 2-8C for short term storage (1 to 2 days) and -20C for long term storage.

APPENDIX A – OPTIONAL DNASE TREATMENT OF EXTRACTED RNA

The truXTRAC FFPE total NA kit isolates total RNA that may contain small amounts of DNA. An optional DNase treatment protocol is provided if DNA-free RNA is desired.

This procedure is done after Step 12 in Section-6 (RNA Purification).

The protocol below describes removal of DNA specifically using TURBO DNA-*free* kit (ThermoFisher Scientific PN AM1907).

1. Prepare a 1 X TURBO DNase master mix:

Table 5 – DNase master mix

Reagent	Volume for N samples*
RNase-free H_2O	**96.8** µl x N
10X TURBO DNase buffer	**11** µl x N
TURBO DNase	**2.2** µl x N

* calculation includes 10% excess in final volume

2. Add 100 µl of DNase master mix to each bead pellet.
3. Re-suspend the beads by pipetting up and down 20 times.
4. Incubate at room temperature for 30 minutes.
5. Add 300 µl of BB3 and vortex for 5 seconds.
6. Place the tube on a magnetic stand and incubate for 5 minutes until the beads have been pulled to the magnet.
7. Carefully remove the supernatant using a 200 µl pipet. Avoid disturbing the bead pellet.
8. Proceed with Step 13 in Section-6 (RNA Purification).

APPENDIX B – TROUBLESHOOTING GUIDE

Issue	Cause	Solution	Comments / Suggestions
Low yield of DNA	First proteinase K incubation too long.	Optimize the 1st proteinase K digestion step for your tissue samples.	During the 1st incubation step with proteinase K at 56C, the RNA is released, and most of the DNA stays in the remaining tissue. If the PK digestion step is too long, the tissue will be over digested resulting in the release of the DNA into the solution.
	Parts or entire tissue pellet lost during supernatant removal.	Repeat using narrow mouth 200 µl pipette tip to take off RNA-containing supernatant.	Follow guidelines in the protocol closely. Make sure laboratory personnel are trained in this procedure.
	Loss of magnetic beads during purification steps.	Remove supernatant of bind and wash steps slowly and carefully. If beads appear in the pipet tip, eject the liquid back into the tube, wait for 1 minute, and try aspirating the supernatant again.	The viscosity of buffers BB3, WB3 and WB4, as well as the presence of the paraffin emulsion can make supernatant removal difficult.
	Low tissue to wax ratio in FFPE section.	Trim off any excess paraffin before sectioning a FFPE tissue block. Repeat the procedure using additional sections until desired yield is achieved.	In your initial use of the truXTRAC FFPE total NA kit use FFPE blocks that have been well characterized for yield and quality.
	Insufficient tissue input.	Select FFPE section with higher tissue to wax ratio or add additional section.	See sample input guidelines in Section-1.
Low yield of RNA	Loss of magnetic beads during purification steps.	Remove supernatant of bind and wash steps slowly and carefully. If beads appear in the pipet tip, eject the liquid back into the tube, wait for 1 minute, and try aspirating the supernatant again.	The viscosity of buffers BB3, WB3 and WB4, as well as the presence of the paraffin emulsion can make supernatant removal difficult.
	Low tissue to wax ratio in FFPE section.	Trim off any excess paraffin before sectioning a FFPE tissue block. Repeat the procedure using additional sections until desired yield is achieved.	In your initial use of the truXTRAC FFPE total NA kit use FFPE blocks that have been well characterized for yield and quality.
Eluates are cloudy	Residual paraffin in elution.	Spin the eluate for 30 seconds at 10,000 rcf. The residual wax will form a layer on top of the liquid and the aqueous solution can be transferred to a new tube.	If the paraffin emulsion was not completely removed in the wash steps, residual wax can be carried through to the elution step.

TIPS FOR DETERMINING QUALITY AND QUANTITY OF THE PURIFIED FFPE DNA/RNA

- To determine DNA and RNA yields, a fluorometric assay such as Qubit ™ (Life Technologies) should be used.
- In addition, spectrophotometric analysis determining the A260/280 and A260/230 ratios will determine if protein or peptide/salt contamination is present in the sample.
- qPCR can be used to assess the amplifiability of isolated DNA as well as the presence of inhibitors. Note that DNA from FFPE tissue itself can act as an inhibitor at high input concentrations due to the extensive damage (e.g., nicks and/or depurination). Therefore, a dilution series over at least 5 orders of magnitude starting with undiluted material of the extracted DNA should always be done when assessing quality by qPCR. An example is shown in Dietrich et al. Figure 1 [3].

ADDITIONAL NOTES

1. See following link: http://covaris.com/resources/protocols/ for updates to this document.
2. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the tissue type, mass, and previous handling of FFPE samples.
3. Covered by US Patent 9,080,167
4. Other patents pending and issued

REFERENCES

1. Carrick et al. (2015) Robustness of Next Generation Sequencing on Older Formalin-Fixed Paraffin-Embedded Tissue. PLoS ONE 10(7): e0127353.
2. Landolt et al. (2016) RNA extraction for RNA sequencing of archival renal tissues. Scand J Clin Lab Invest 76(5):426-434.
3. Dietrich et al. (2013) Improved PCR Performance Using Template DNA from Formalin-Fixed and Paraffin-Embedded Tissues by Overcoming PCR Inhibition. PLOS one 8(10):e77771