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truXTRAC® FFPE total NA (tNA) Ultra Kit – Magnetic Bead (25)

Adaptive Focused Acoustics® (AFA®)-based
Sequential RNA and DNA Extraction from FFPE Tissues

PN 520304

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General Information

Intended Use

The truXTRAC FFPE total NA (tNA) Ultra Kit - Magnetic Bead (PN 520304) 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 (tNA) Ultra Kit - Magnetic Bead 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 20 µm of total FFPE tissue sections. For sample inputs that differ from the requirements or for very small inputs, please contact ApplicationSupport@covaris.com.

This protocol enables manual sequential purification of RNA and DNA from FFPE samples. In this protocol, RNA and DNA are purified separately using two 2 ml microcentrifuge tubes per sample (one for RNA and one for DNA). The user will manually add and remove buffers to purify the nucleic acids using magnetic bead technology.

Important Notes on FFPE Samples: The yield of DNA and RNA from FFPE tissue blocks is highly variable. Factors such as fixation time, 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.

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

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Revision History

Part Number	Revision	Date	Description of Change
010533	A	8/2020	Initial Release
010533	B	3/2021	No major protocol changes. Correcting typographical errors.

Kit Contents

- Tissue Lysis Buffer .... 25 ml
- Proteinase K (PK Solution).. 3.5 ml
- Magnetic Bead Suspension .... 0.5 ml
- Buffer BB3 .... 45 ml
- Buffer WB3.. 2 x 60 ml
- RNA Elution Buffer.. 3.5 ml
- Buffer BE.. 7.5 ml
- AFA-TUBE PP Screw-Cap 0.5 ml 25

SDS Information available at: www.covaris.com/safety-data-sheets/

Storage

Upon kit arrival, store the Proteinase K Solution and the Magnetic Bead Suspension at 2 to 8 °C. Store all other kit components at ambient temperature.

Laboratory Equipment, Chemicals, and Consumables Supplied by User

Required Laboratory Equipment and Accessories

- Magnetic Rack for 2 ml microcentrifuge tubes (Thermofisher, PN 12321D)
- 0.5 ml Centrifuge Adapters (Eppendorf, PN 022636227)
- Dry block heater with blocks to accommodate 2 ml tubes or temperature-controlled water bath able to accurately heat between 50 to 90 °C
- Dry block heater with blocks to accommodate 0.5 ml microcentrifuge conical tubes or temperature-controlled water bath able to accurately heat between 50 to 90 °C

Required Chemicals and Enzymes

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

Optional Enzymes

- TURBO DNase (2 U/µL) (Thermo Fisher Scientific, PN AM2238), containing 10X TURBO DNase Buffer
- DNase-free RNase A (10 mg/ml) (e.g., Thermo Fisher Scientific, PN EN0531)

Required Consumables

- Eppendorf tubes 2 ml (Eppendorf, PN 0022363344)
- Tubes to make buffer mixtures

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.

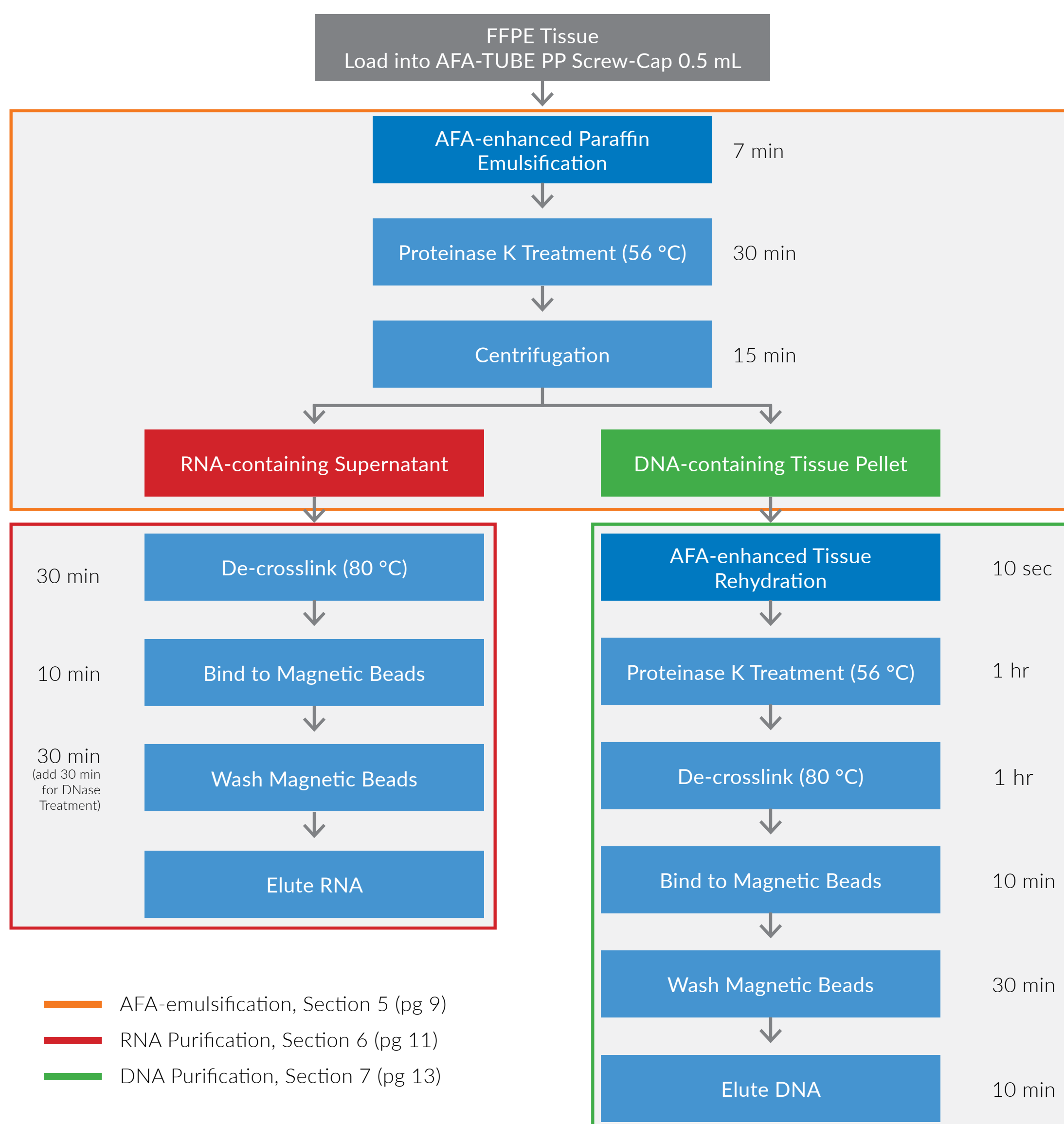
Instrument	ML230	M220
Holder/Rack Description (PN)	ML230 Rack 6 Place AFA-TUBE PP Screw-Cap 0.5 ml (PN 500696)	Holder XTU (PN 500414)
Plate Definition File Name	<500696 Rack 6 Place AFA-TUBE PP Screw-Cap 0.5 ml>	N/A
Required Accessories (PN)	N/A	XTU Insert (PN 500692)

FFPE tNA Extraction and Purification Workflow

Using the Adaptive Focused Acoustics (AFA) process, FFPE tissue samples are prepared in Tissue Lysis Buffer in the presence of Proteinase K, followed by an incubation at 56 °C 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.

The DNA-containing tissue is re-suspended through AFA, and then digested with Proteinase K, releasing the DNA. The released DNA is de-crosslinked through heated incubation, followed by purification.



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:

Slide Collection Method	Maximum Input per AFA-TUBE PP Screw-Cap 0.5 ml
Scalpel or razor blade to scrape material from slides	20 µm of total thickness (4 slides at 5 µm thick = 20 µm total thickness) Max Area (on each slide): 10 mm x 10 mm

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 Curl/Scroll Thickness	Maximum Scrolls per AFA-TUBE PP Screw-Cap 0.5 ml
5 µm	4
10 µm	2
15 µm	1

For inputs not listed, please contact ApplicationSupport@covaris.com.

2 - Preparation of Reagents

Follow these instructions before starting the FFPE tNA isolation protocol.

1. ***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 to 60 °C 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 2.6 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 2.9 ml (includes 10% extra volume).

3 - Preparation of Heat Blocks

1. Preheat dry block heaters to 56 °C and 80 °C ± 3 °C. It is critical that these temperatures are accurate in order to successfully execute the protocol.
2. Test the temperature of your heat blocks:
   a. Place a 0.5 ml microcentrifuge tube filled with water into the heat block for 0.5 ml tubes, and a 2 ml microcentrifuge tube filled with water into the heat block for 2 ml tubes.
   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 56 °C ± 2 °C or 80 °C ± 2 °C.

> **CAUTION:** The AFA-TUBE PP Screw-Cap 0.5 ml must be used in conjunction with a compatible heat block, such as, Eppendorf SmartBlock™ 0.5 mL, thermoblock for 24 reaction vessels 0.5 mL, incl. Transfer Rack 0.5 mL (Eppendorf, Cat No. 5361000031). It is important to use an accurate heating source for incubation of AFA-TUBE PP Screw-Cap 0.5 ml and microcentrifuge tubes during Proteinase K and de-crosslinking incubations. Deviation from 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 at 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	ML230	M220
Peak Incident Power (PIP) (Watt)	330	50
Duty Factor (%)	30	15
Cycles Per Burst (CPB)	1000	800
Treatment time (seconds)	480	300
Bath temperature (C)	20	20
Water Level (run)	Automatic	Full

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	ML230	M220
Peak Incident Power (PIP) (Watt)	330	50
Duty Factor (%)	30	15
Cycles Per Burst (CPB)	1000	800
Treatment time (seconds)	10	10
Bath temperature (C)	20	20
Water Level (run)	Automatic	Full

5 - Paraffin Emulsification, Tissue Rehydration, and Lysis

1. Prepare 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 Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min of preparation.

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	510 µl	510 µl x N
Proteinase K Solution	40 µl	40 µl x N

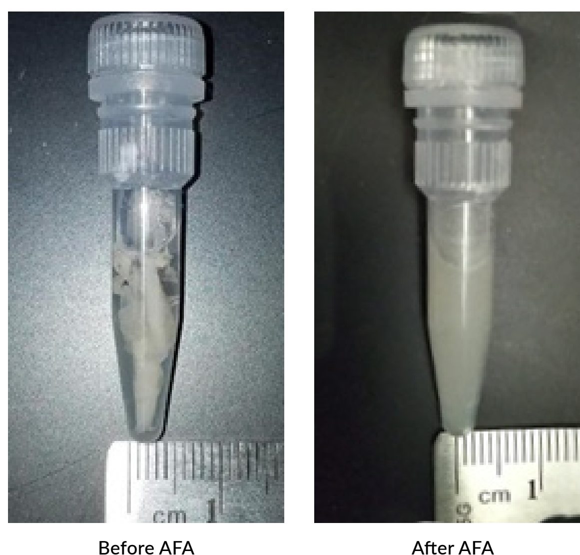
Table 1. Tissue Lysis Buffer /Proteinase K Mix. *Calculation includes 10% excess in final volume

2. Open the AFA-TUBE PP Screw-Cap 0.5 ml and load the FFPE tissue to the bottom of the tube.
3. Add 500 µl Tissue Lysis Buffer/Proteinase K Mix to AFA-TUBE PP Screw-Cap 0.5 ml.
4. Make sure that the tissue is completely submerged in Tissue Lysis Buffer/Proteinase K Mix.
5. Close the AFA-TUBE PP Screw-Cap 0.5 ml tightly with the Screw-Cap and transfer the AFA-TUBE PP Screw-Cap 0.5 ml to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the tube(s) into the Focused-ultrasonicator for processing.

CAUTION: If treating less than 6 samples on the ML230, load AFA-TUBE PP Screw-Cap 0.5 ml containing water in the remaining rack positions to prevent splashing.

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

NOTE: It is expected that the solution will turn milky white.



Before AFA After AFA

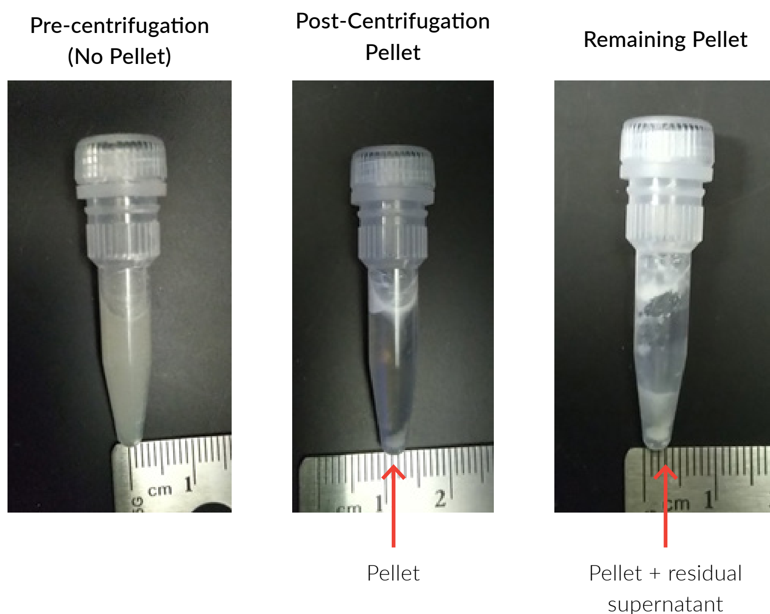
5 - Paraffin Emulsification, Tissue Rehydration, and Lysis (cont.)

7. Remove the AFA-TUBE PP Screw-Cap 0.5 ml from the Focused-ultrasonicator, and load the tube into the 0.5 ml Heat Block set to 56 °C. When processing in batches, samples may be kept at room temperature for up to two hours prior to Proteinase K incubation at 56 °C (***Step 7***).
8. Incubate for 30 minutes at 56 °C. Remove AFA-TUBE PP Screw-Cap 0.5 ml and let it cool at room temperature for 3 min.
9. Place AFA-TUBE PP Screw-Cap 0.5 ml with 0.5 ml tube centrifuge adapters into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 15 minutes.

> **NOTE:** The inner centrifuge lid might not close and may need to be left off during centrifugation.

10. Open the AFA-TUBE PP Screw-Cap 0.5 ml and carefully transfer 450 µl RNA 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 at the bottom of the tube with more pellet toward the side 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 aspirate the supernatant while simultaneously lowering the tip following the liquid level. Place the pipette tip 2 to 3 mm above the pellet, towards the tube wall that faces away from the pellet. Use the same 200 µl pipette tip for a total of 3 times 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 approximately 50 µl of supernatant behind. This will not significantly impact RNA or DNA yield.



> **NOTE:** If the pellet becomes dislodged from the bottom of the AFA-TUBE PP Screw-Cap 0.5 ml before the RNA supernatant has been removed, repeat centrifugation (Step 9) to re-pellet the DNA. Remove RNA supernatant as described in Step 10.

11. 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 to 8 °C for up to 1 day. For longer periods, store at –15 to –30 °C.

6 - FPPE RNA Purification

The protocol allows purification of RNA with an option for DNase treatment after Step 12 (***See Appendix B***).

1. Set up the 2 ml dry-heat block heaters as explained in ***Section-3*** to 56 C (Step 6) and 80 C (***Step 2***).
2. Incubate 2 ml microcentrifuge tube with the RNA-containing supernatant at 80 C for 30 minutes. Remove tubes from the heat block and cool at room temperature for 3 minutes.
3. Prepare BB3/Magnetic Bead Mix according to ***Table 2*** below.

Reagent	Volume for one sample*	Volume for N samples*
BB3	607 µl	607 µl x N
Magnetic Bead Suspension	8.8 µl	8.8 µl x N

Table 2. BB3/Magnetic Bead Mix For RNA. *Calculation includes 10% excess in final volume

CAUTION: Thoroughly vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using.

4. Add 560 µl of BB3/Magnetic Bead Mix to the RNA-containing supernatant and cap the microcentrifuge tube.
5. Vortex the microcentrifuge tube for 5 seconds.
6. Incubate the microcentrifuge tube at 56 °C 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.

NOTE: 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 and discard the supernatant using a 200 µl pipette. Avoid touching or disturbing the bead pellet.
9. Remove the microcentrifuge tube from the magnetic stand and add 1 ml WB3.
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 microcentrifuge tubes on the magnet, carefully remove and discard the supernatant.
    Optional DNA removal step: The truXTRAC FFPE total NA (tNA) Ultra Kit – Magnetic Bead 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 B*** for step-by-step instructions.
13. Repeat steps 9 through 12 for the second wash.

6 - RNA Purification (cont.)

14. After the second wash, remove as much of the supernatant as possible. Use a 20 µl pipettor to remove the remaining liquid from the bottom of the microcentrifuge tubes.

:

> **NOTE:** 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.

15. Remove the tube from the magnetic stand and add 1 ml 80% ethanol.
16. 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.

> **NOTE:** With some samples, beads may stick to the tube wall and not completely resuspend during vortexing. Proceed to the next step of the protocol even if the beads are not completely resuspended.

17. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.
18. Remove and discard the supernatant without disturbing the bead pellet.
19. Remove the tube from the magnetic stand and add 300 µl 80% ethanol.
20. Cap the tube and vortex for 10 seconds.
21. Place the microcentrifuge tube on the magnetic stand and wait for 2 minutes, until the beads have been pulled to the magnet.
22. Remove and discard as much of the supernatant as possible. Use a 20 µl pipette to remove the remaining liquid from the bottom of the microcentrifuge tube.
23. Leave the microcentrifuge tube uncapped on the magnetic stand for 6 minutes at ambient temperature to let the beads dry.

> **NOTE:** Visually examine that the ethanol has evaporated before continuing with elution. Residual ethanol can inhibit the elution and impact downstream applications such as PCR.

24. Remove the microcentrifuge tubes from the magnetic stand and add 50 to 100 µl of the RNA Elution Buffer. Resuspend the beads by pipetting up and down 20 times. Ensure that all beads are resuspended in the buffer.
25. Cap the microcentrifuge tube and incubate in the heat block set to 56 °C for 5 minutes.
26. Transfer the microcentrifuge tubes from the heat block to the magnetic stand and incubate for 2 minutes.
27. Transfer the eluate into a new/clean microcentrifuge tube without transferring beads. A small amount of residual paraffin may be visible in the pipette tip. This will not adversely affect downstream processing of the eluted RNA.
28. Store the eluted RNA on ice until further processing. Isolated RNA should be kept at -80 °C for long term storage.

7 - FPPE DNA Purification

1. Preheat dry block heaters with 0.5 ml heat blocks to 56 °C (Steps 7 and 15) and 80 °C (Step 9) ± 2 °C.
2. Prepare 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 Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min after preparation.

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

Table 3. Tissue Lysis Buffer/Proteinase K Mix for DNA. *Calculation includes 10% excess in final volume

3. Open the AFA-TUBE PP Screw-Cap 0.5 ml with the DNA-containing tissue pellet and add 400 µl of the Tissue Lysis Buffer/Proteinase K Mix for DNA.
4. Close the AFA-TUBE PP Screw-Cap 0.5 ml tightly with the Screw-Cap and transfer the AFA-TUBE PP Screw-Cap 0.5 ml to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the AFA-TUBE PP Screw-Cap 0.5 ml into the Focused-ultrasonicator for processing.

CAUTION: If treating less than 6 samples on ML230, load AFA-TUBE PP Screw-Cap 0.5 ml containing water in the remaining rack positions to prevent splashing.

5. Process the sample using the “Acoustic Pellet Resuspension” program on your Focused-ultrasonicator.
6. Remove the AFA-TUBE PP Screw-Cap 0.5 ml from the Focused-ultrasonicator and load it into the 0.5 ml heat block set to 56 °C.
7. Incubate for a minimum of 60 minutes at 56 °C.

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

8. Remove AFA-TUBE PP Screw-Cap 0.5 ml from the heat block and transfer directly to dry heat block set-up for 80 °C incubation.
9. Incubate for 60 minutes at 80 °C.
10. Remove the AFA-TUBE PP Screw-Cap 0.5 ml 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 Table 4 below.

Reagent	Volume for one sample*	Volume for N samples*
BB3	607 µl	607 µl x N
Magnetic Bead Suspension	8.8 µl	8.8 µl x N

Table 4. BB3/Magnetic Bead Mix For DNA. *Calculation includes 10% excess in final volume

CAUTION: Thoroughly vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using.

13. Add 560 µl of BB3/Magnetic Bead Mix to the DNA containing supernatant and cap the microcentrifuge tube.
14. Vortex the microcentrifuge tube for 5 seconds.
15. Incubate the microcentrifuge tube at 56 °C for 5 minutes.
16. Place the microcentrifuge tubes on a magnetic stand and wait for 5 minutes or until the beads have been pulled to the magnet.

NOTE: 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 microcentrifuge tubes still on the magnet, carefully remove and discard the supernatant. Avoid disturbing the bead pellet.
18. Remove microcentrifuge tubes from the magnetic stand and add 1 ml of the Buffer WB3.
19. Cap the microcentrifuge tubes and vortex for 10 seconds to resuspend the beads.

NOTE: With some samples, beads may stick to the tube wall and not completely resuspend during vortexing. Proceed to the next step of the protocol even if the beads are not completely resuspended.

20. Place the microcentrifuge tubes back on the magnet stand and wait for 5 minutes until the beads have been pulled to the magnet.
21. With the microcentrifuge tubes on the magnet, carefully remove and discard the supernatant.
22. Repeat wash steps 18 through 21.
23. 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.

NOTE: 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.

24. Remove the microcentrifuge tubes from the magnetic stand and add 1 ml of the 80% ethanol solution to the tubes.
25. Cap the microcentrifuge tubes and vortex for 10 seconds to resuspend the beads.

NOTE: With some samples, beads may stick to the tube wall and not completely resuspend during vortexing. Proceed to the next step of the protocol even if the beads are not completely resuspended.

26. Place the microcentrifuge tubes on the magnetic stand and wait for 2 minutes, until the beads have been pulled to the magnet.
27. Remove and discard the supernatant without disturbing the bead pellet.
28. Remove the microcentrifuge tubes from the magnetic stand and add 300 µl of the 80% ethanol solution.
29. Cap the microcentrifuge tubes and vortex for 10 seconds.
30. Place the microcentrifuge tubes on the magnetic stand and wait for 2 minutes, until the beads have been pulled to the magnet.
31. Remove and discard as much of the supernatant as possible. Use a 20 µl pipette to remove the remaining liquid from the bottom of the tube.
32. Leave the microcentrifuge tubes uncapped on the magnetic stand and let the beads dry for 6 minutes at ambient temperature.

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

33. Remove the microcentrifuge tubes from the magnetic stand and add 50 to 100 µl of Buffer BE (5 mM TrisCl pH 8.5) into the tube.
34. Re-suspend the beads by pipetting up and down 20 times. Ensure that all beads are submerged in the buffer and are fully suspended.
35. Cap the microcentrifuge tubes and incubate them in the heat block set to 56 °C for 5 minutes.
36. Remove the microcentrifuge tubes from the heat block and place it on the magnetic stand and wait for 2 minutes, until the beads have been pulled to the magnet.
37. Transfer the eluate into a clean/new microcentrifuge tube avoiding transfer of beads. A small amount of residual paraffin may be visible in the pipette tip. This will not adversely affect downstream processing of the eluted DNA.
38. Isolated DNA should be kept at 2 °C to 8 °C for short term storage (1 to 2 days) and −20 °C for long term storage.

Appendix A - 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 56 °C, 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.	Pipette from the top of the liquid surface and lower the tip as the volume decreases using a narrow pipette tip.
	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 (tNA) Ultra Kit - Magnetic Bead, 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	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 (tNA) Ultra Kit - Magnetic Bead 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.
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.

Appendix B - Optional DNase Treatment of Extracted RNA

The truXTRAC FFPE total NA (tNA) Ultra Kit – Magnetic Bead isolates total RNA which 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 DNase (2 U/µL) (Thermo Fisher Scientific, PN AM2238), containing 10X TURBO DNase Buffer.

1. Prepare a 1X TURBO DNase master mix:

Reagent	Volume for N samples*
RNase-free H2O	93.5 µL x N
10X TURBO DNase buffer	11 µL x N
TURBO DNase	5.5 µL x N

Table 5. DNase Master Mix. *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 ambient temperature for 30 minutes.
5. Add 300 µl of Buffer BB3 and vortex for 5 seconds.
6. Incubate at ambient temperature for 10 minutes.
7. Place the microcentrifuge tubes on a magnetic stand and incubate for 5 minutes or until the beads have been pulled to the magnet.
8. Carefully remove the supernatant using a 200 µl pipette. Avoid disturbing the bead pellet.
9. Proceed with ***Step 13*** in ***Section-6*** (RNA Purification).

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Tips for Determining Quality and Quantity of the Purified FFPE 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 inhibitor at high input concentrations due to the extensive damage (nicks, depurination, etc.). 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].
- Fragment size analysis of the RNA can be used to estimate the quality of the RNA by comparing the %DV200 [4, 5]
- RT-qPCR can be used to assess the amplifiability of the isolated RNA and correlated to the success of sequencing. [5]

Additional Notes

1. See following link: www.covaris.com/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

References

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