

truXTRAC[®] FFPE DNA Plus Kit – Magnetic Beads

Adaptive Focused Acoustics[®] (AFA[®])-based DNA Extraction
from FFPE Tissues using Magnetic Bead-based Purification

PN 520262

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General Information

Intended Use

The truXTRAC FFPE DNA Plus Kit – Magnetic Beads is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of any disease.

Introduction

The truXTRAC FFPE DNA Plus Kit is designed for efficient and sequential extraction of 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 DNA for sensitive analytical methods such as next-generation sequencing (NGS) or qPCR/RT-qPCR. Finally, the workflow is simple in that it does not require any centrifugation steps.

This protocol is optimized for up to 20 µm of total FFPE tissue sections or two FFPE cores (1.2 mm in diameter and 5 mm in length).

This protocol includes optional instructions for storage of FFPE samples in mineral oil. See **Appendix B** for more information.

Important Notes on FFPE Samples:

The yield of DNA 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 isolated from FFPE samples can also be highly variable. During the fixation process, DNA 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 [2].

Note for users:

If you require any assistance with this product, please refer to Troubleshooting (**Appendix C**) 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 |
|-------------|----------|-------|---|
| 010466 | A | 8/18 | Initial Release of truXTRAC FFPE DNA Plus Kit – Magnetic Beads |
| 010466 | B | 10/18 | Format modification and layout improvement |
| 010466 | C | 3/19 | Updated Appendix A for Sonolab 8. Added Appendix B with instructions for mineral oil storage and removal. |
| 010466 | D | 8/19 | Remove tissuePICK, sectionPICK and related accessories |
| 010466 | E | 7/20 | Update Kit Contents typo |

Kit Contents

- DNA Lysis Buffer..... 12 ml
- Proteinase K (PK Solution) 2.4 ml
- Magnetic Bead Suspension..... 0.24 ml
- Buffer BB3 25 ml
- Buffer WB3..... 60 ml
- Buffer BE..... 3 ml
- microTUBE-500 AFA Fiber Screw-Cap FFPE ... 25 tubes

SDS information is available at: www.covaris.com/resources/safety-data-sheets/

Storage

Upon kit arrival, store the Proteinase K solution and the Magnetic Bead Suspension at 2 °C to 8 °C. Store all other kit components at ambient 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 °C to 90 °C
- Magnet Stand for 2 ml tubes (e.g. Thermo Fisher Scientific, DynaMag-2 Magnet, PN 12321D)

Required Chemical and Enzymes

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

Optional Enzymes

- RNase A, DNase and protease-free (10 mg/ml) (e.g., Thermo Fisher Scientific, PN EN0531)

Required Consumable

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

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.

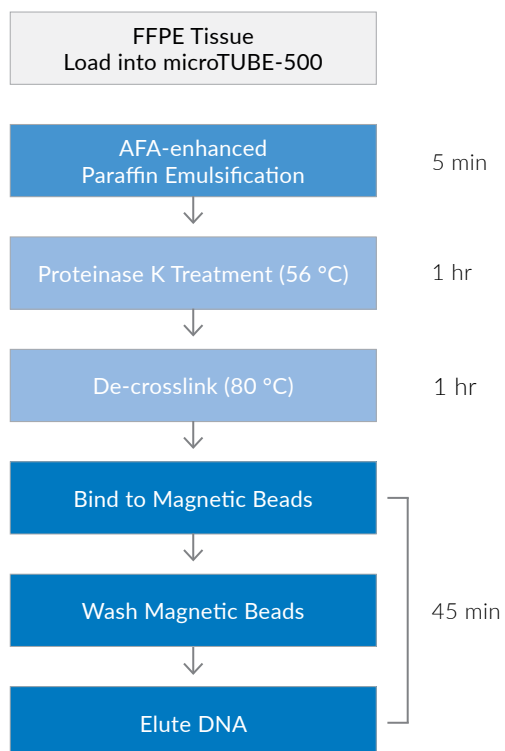
| Instrument | M220 | ME220 | S220 | E220 <i>evolution</i> | E220 | LE220/LE220-plus |
|------------------------------|---------------------|---|--|---|--|--|
| Holder/Rack Description (PN) | Holder XTU (500414) | Rack 4 Position micro-TUBE-500 (500525) | Holder micro-TUBE-500 Screw Cap (500449) | Rack 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 | N/A | <4 microTUBE-500 Screw-Cap PN 520185> | N/A | <500484 E220e 4 microTUBE-500 Screw-Cap> | <500452 Rack 24 Place microTUBE-500 Screw-Cap> | <500452-24 micro-TUBE-500 Screw-Cap +6mm offset z enabled> |
| Required Accessories (PN) | Insert XTU (500471) | ME220 Waveguide 4 Place (500534) | N/A | Intensifier (500141) | Intensifier (500141) | N/A |

Please find the LE220 and LE220-plus plate definition files here: <https://covaris.com/products/truxtrac-ffpe-dna-and-rna-kits/truxtrac-ffpe-dna/#toggle-id-3>

FFPE DNA Extraction and Purification Workflow

Prior to loading, FFPE samples can be stored in mineral oil. See **Appendix B** for detailed information.

Using the Adaptive Focused Acoustics (AFA) process, FFPE samples are prepared in DNA Lysis Buffer in the presence of Proteinase K. DNA is released from the tissue by AFA-enhanced Proteinase K digestion, followed by a de-crosslinking step. DNA is then purified using magnetic beads.



1 - FFPE Sample Input Requirements and Guidelines

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

Slide Section Input Requirements:

| Slide Collection Method | Maximum Input per microTUBE-500 |
|---|---|
| Scalpel or razor blade to scrape material from slides | 30 μm of total thickness (e.g., 6 slides at 5 μm thick = 30 μm total thickness) Max Area (on each slide): 10 mm^2 |

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.

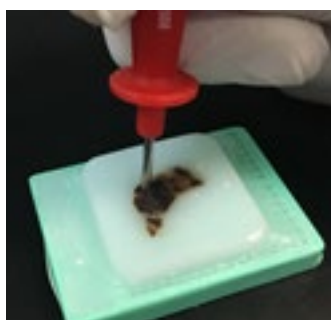
| FFPE Curl/Scroll Thickness | Maximum Scrolls per microTUBE-500 |
|----------------------------|-----------------------------------|
| 5 μm | 4 |
| 10 μm | 2 |
| 15 μm | 1 |

Core Input Requirements:

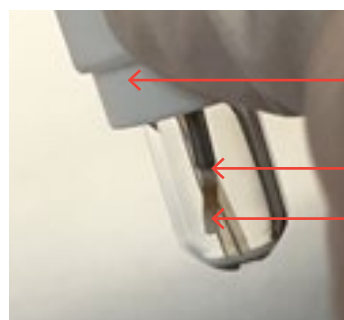
| FFPE Core Punch Outer Diameter | Maximum Core Punches per microTUBE-500 |
|---|--|
| \leq 1.2mm (15 Gauge, outer); Length = 5 mm | 2 |

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

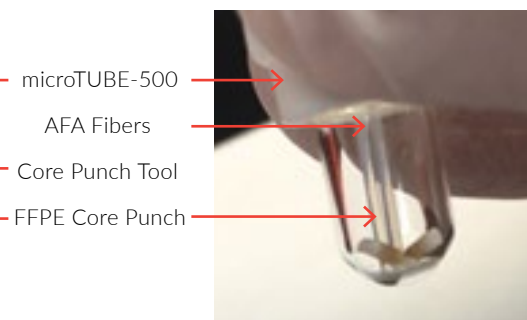
**Core Punch from
FFPE Block**



**Loading Core Punch
into microTUBE-500**



**Core Punches Loaded
into microTUBE-500**



2 - Preparation of Reagents

NOTE: Follow these instructions before starting the FFPE DNA isolation protocol.

1. **DNA Lysis Buffer:** Visually check for a white precipitate that may form during storage before each use. If white precipitate is visible, incubate the DNA Lysis Buffer at 50 °C to 60 °C for 5 to 10 minutes before use to dissolve any precipitate.
2. **80% Ethanol Solution:** Prepare the 80% ethanol solution by mixing 4 parts 100% ethanol with 1 part nuclease-free water. One sample requires 1.3 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 1.5 ml to account for dead volume.

3 - Preparation of Heat Blocks

1. Preheat dry block heaters to 56 °C and 80 °C ± 2 °C. It is critical that these temperatures are accurate to successfully execute the protocol.
2. To test the temperature of your heat blocks:
 - a. Place a heat block adapter and a microTUBE-500 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 microTUBE-500 has reached 56 °C or 80 °C ± 2 °C.

CAUTION: The microTUBE-500 must be used in conjunction with microTUBE-500 Centrifuge and Heat Block Adapters (PN 500503).

It is important to use an accurate heating source for incubation of microTUBE-500 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)

NOTE: 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 the “Acoustic Paraffin Emulsification” program using the Covaris SonoLab method editor. Save the program for later use.

Refer to **Appendix A** for instructions on adding dithering parameters to the method.

| Instrument | M220 | ME220 | S220 | E220evolution | E220 | LE220/LE220-plus |
|--|------|---|------|---------------|------|---|
| Peak Incident Power (PIP) (Watt) | 75 | 75 | 200 | 200 | 200 | 450 |
| Duty Factor (%) | 25 | 25 | 10 | 10 | 10 | 20 |
| Cycles Per Burst (CPB) | 200 | 1000 | 200 | 200 | 200 | 200 |
| Treatment time (seconds) | 360 | 300 | 300 | 300 | 300 | 300 |
| Bath temperature (C) | 20 | 20 | 20 | 20 | 20 | 20 |
| Dithering (See Appendix A for dithering setup) | N/A | X Dither: 1 mm Y Dither: 2 mm Dither Speed: 10 mm/sec | N/A | N/A | N/A | X Dither: 1.5 mm Y Dither: 3 mm Dither Speed: 10 mm/sec |
| Water Level (run) | Full | Auto | 8 | 6 | 6 | 6 |

Paraffin Emulsification, Tissue Rehydration, and Lysis

5 - Paraffin Emulsification, Tissue Rehydration, and Lysis

NOTE: If samples were stored in mineral oil, follow **Appendix B** on proper removal prior to continuing.

1. Prepare the DNA Lysis Buffer/PK Solution Mix by following instructions in **Table 1** below. Mix by inverting 10 times or vortexing for 3 seconds.

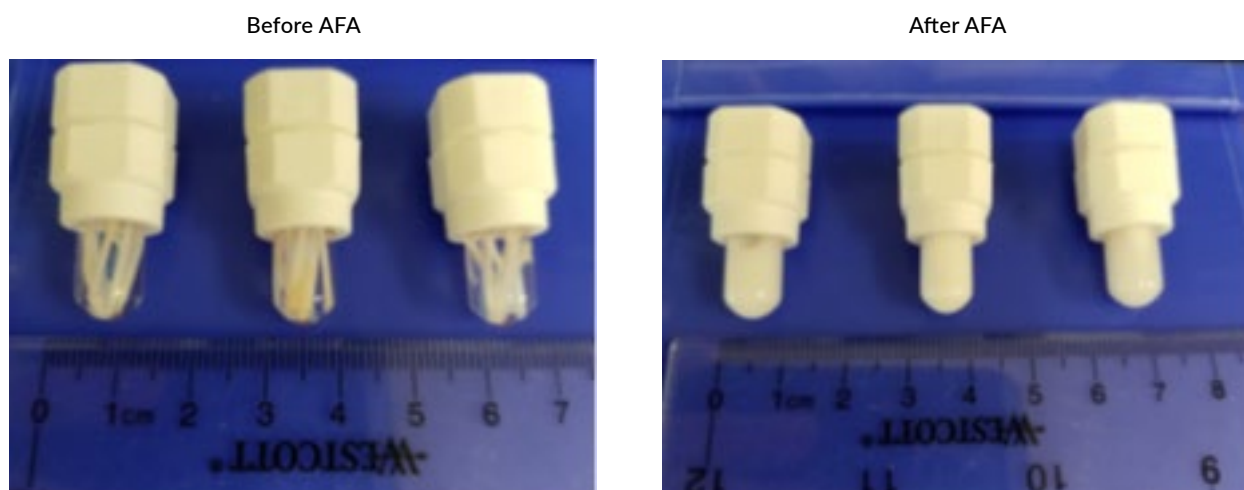
CAUTION: The DNA Lysis Buffer/PK Solution Mix should be stored at ambient temperature and used within 30 min of preparation.

| Reagent | Volume for One Sample* | Volume for N Samples* |
|------------------|------------------------|-----------------------|
| DNA Lysis Buffer | 396 μ l | 396 μ l x N |
| PK Solution | 88 μ l | 88 μ l x N |

Table 1. DNA Lysis Buffer/PK Solution Mix. *Calculation includes 10% excess in final volume.

2. Load each FFPE sample into a microTUBE-500.
3. Add 440 μ l of the DNA Lysis Buffer/PK Solution Mix into each microTUBE-500. 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-500s tightly with their Screw-Caps and transfer the microTUBE-500s to the appropriate rack or holder/insert for your Focused-ultrasonicator.
5. Load the rack or holder/insert containing the microTUBE-500s into the Focused-ultrasonicator for processing.
6. Process the sample using the “**Acoustic Paraffin Emulsification**” program.

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



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

- Transfer the microTUBE-500s from the Focused-ultrasonicator to the microTUBE-500 Centrifuge and Heat Block Adapters on the heat block set to 56 °C.
- 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.

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

- Remove the microTUBE-500s with the adapters from the heat block and transfer directly to dry heat block set for 80 °C incubation.
- Incubate for 60 minutes at 80 °C.
- Remove the microTUBE-500s with the adapters from the heat block and let cool for 3 minutes at ambient temperature.
- Transfer the sample to a 2 ml microcentrifuge tube.

NOTE: 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 ambient temperature, then continue to Section-6 for DNA purification.

DNA Purification

6 - DNA Purification

1. Verify that the heat block is set to 56 °C (Steps 5 and 25).
2. Prepare the BB3/Magnetic Bead Mix according to the **Table 2**.

| 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 |

Table 2. BB3/Magnetic Bead Mix. *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.

3. Add 728 µl of the BB3/Magnetic Bead Mix to the DNA solution in the 2 ml microcentrifuge tube.
4. Cap the microcentrifuge tubes and vortex for 10 seconds.
5. Incubate the microcentrifuge tubes at 56 °C for 5 minutes.
6. Place the microcentrifuge tubes on a magnetic stand and wait for 5 minutes or 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.

7. With the microcentrifuge tubes still on the magnet, carefully remove and discard the supernatant. Avoid disturbing the bead pellet.
8. Remove the microcentrifuge tubes from the magnetic stand and add 1 ml Buffer WB3.
9. Cap the microcentrifuge tubes 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.
10. Place the microcentrifuge tubes back on the magnet stand and wait for 5 minutes, until the beads have been pulled to the magnet.
11. With the microcentrifuge tubes on the magnet, carefully remove and discard the supernatant.
12. Repeat wash steps 8 through 11.
13. 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 tubes.

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.

6 - DNA Purification (cont.)

14. Remove the microcentrifuge tubes from the magnetic stand and add 1 ml 80% ethanol solution to the tubes.
15. Cap the microcentrifuge tubes 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.
16. Place the microcentrifuge tubes back on the magnet stand and wait for 2 minutes, until the beads have been pulled to the magnet.
17. With the microcentrifuge tubes on the magnet, carefully remove and discard the supernatant.
18. Remove the microcentrifuge tubes from the magnetic stand and add 300 µl 80% ethanol solution.
19. Cap the microcentrifuge tubes and vortex for 10 seconds. Confirm that all beads are resuspended.
20. Place the microcentrifuge tubes on the magnetic stand and wait for 2 minutes, until the beads have been pulled to the magnet.
21. 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.
22. Leave the microcentrifuge tubes uncapped on the magnetic stand and let the beads dry for 8 minutes at ambient temperature.

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 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.

NOTE: 50 µl of Buffer BE should be used if high DNA concentration is desired and 100 µl should be used for maximal DNA yield.

24. Re-suspend the beads by pipetting up and down 20 times. Ensure that all beads are submerged in the buffer and are fully suspended.
25. Cap the microcentrifuge tubes and incubate them in the heat block set to 56 °C for 5 minutes.
26. 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.
27. Transfer the eluate into a clean/new microcentrifuge tube avoiding transfer of 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.
28. 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.

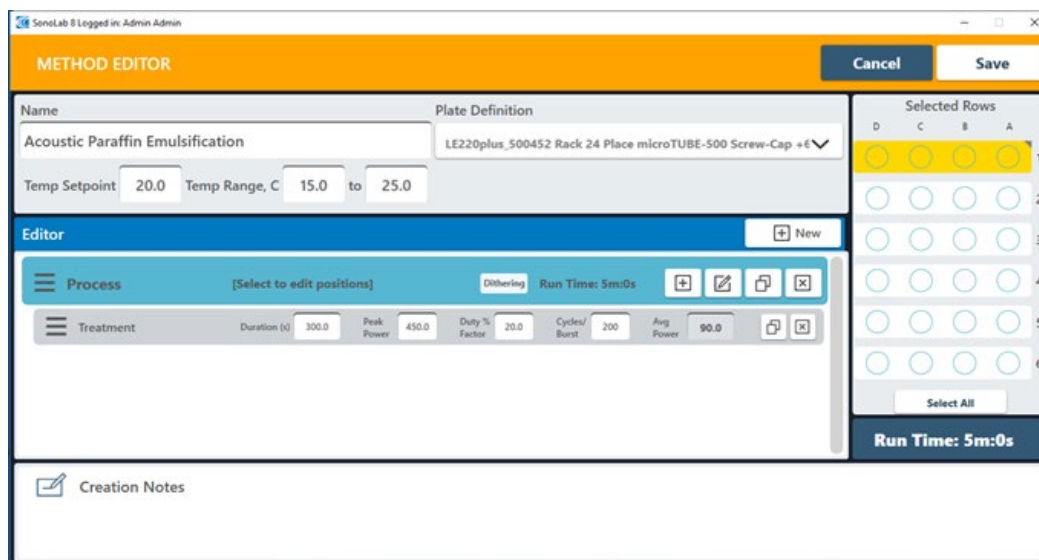
Appendix

Appendix A - Protocol Creation in SonoLab

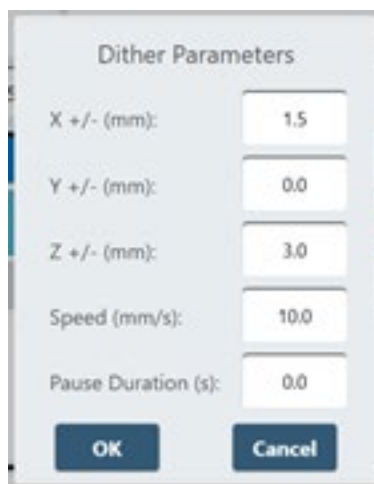
LE220-plus with SonoLab 8.4 or Higher

Refer to the LE220-plus Manual: www.covaris.com/wp-content/uploads/pn_010398.pdf

1. On the Home screen, click the “New” button to bring up the Method Editor.
2. In the Method Editor, set up the new method as pictured below.



3. For Dithering: Click the “Dither Parameter” button in the Process Editor, input the X, Z and Speed dithering values shown below. Then click the “OK” button.

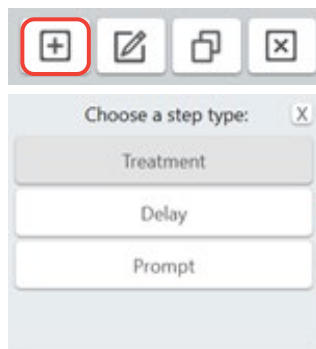


4. Edit the remaining parameters to match the Method Editor screenshot above.
5. Select the sample rows to run.
6. Click the “Save” button once finished.

ME220 with SonoLab 8

Refer to the ME220 User Manual: www.covaris.com/wp-content/uploads/pn_010325.pdf

1. Click the “New” button from the main screen.
2. Input the protocol name as “*Acoustic Paraffin Emulsification*”.
3. Select the <4 microTUBE-500 Screw-Cap PN 520185> Rack Definition from the drop-down menu.
4. Click the “+ New Item” (Add New Process or Repeat) button.
5. In the Process Bar click “Add a step to the process” and then select “Treatment”.



6. Change the AFA settings in the Treatment bar as shown below.



7. Click the “Edit Process Settings” button.



8. Input the X- and Z-dithering values and then click “OK”.



9. In the Editor box, select the number of samples to run in the Process bar.
10. Click the “Save” button once finished.

Appendix B - Pre-treatment for Mineral Oil Stabilized FFPE (Optional)

Introduction

The Pre-treatment for Mineral Oil-Stabilized FFPE is specifically designed to prepare FFPE tissue samples such as scrolls, slide scrapings and cores that are submerged in mineral oil for downstream extraction of nucleic acids. Once a FFPE sample is cut from the main block, storage in mineral oil can prevent unwanted oxidation of biomolecules during storage and shipment. Thus, the sample can be shipped or stored safely until extraction of biomolecules at the laboratory site. The mineral oil removal workflow also ensures that excessive wax/paraffin is partially removed, thereby reducing the need for tedious trimming of wax from the tissue block.

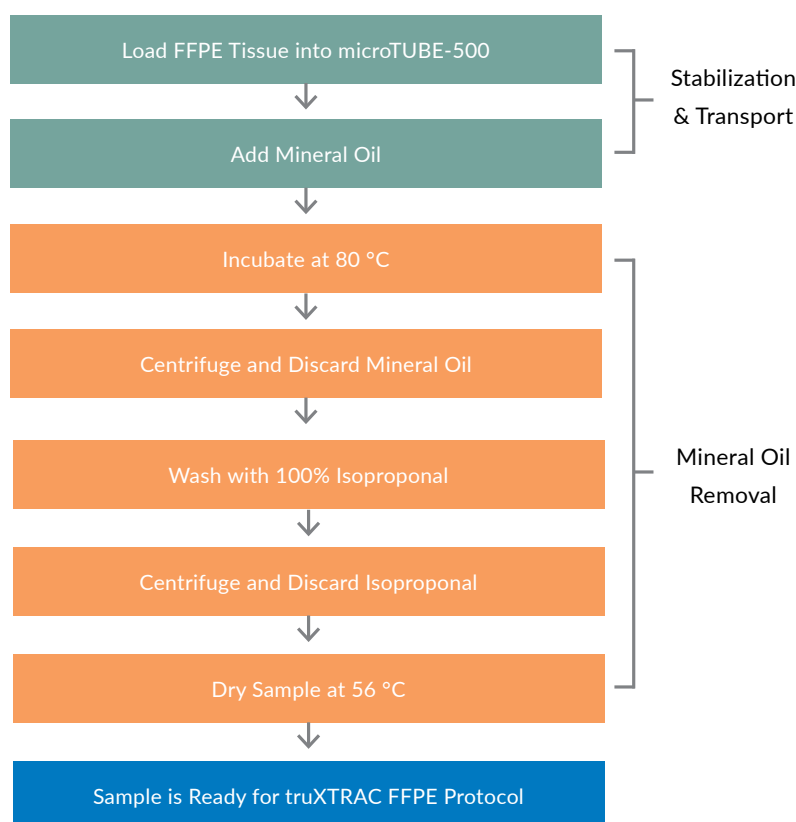
This protocol is fully compatible with downstream extraction of nucleic acids from Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples using Covaris Adaptive Focused Acoustics (AFA).

Note that the FFPE sample input requirements are different than the standard, no mineral oil preserved samples. The maximal amount of tissue per microtube increases with the addition of mineral oil.

Required Additional Reagents

- Mineral Oil, light oil for molecular biology (e.g. Sigma-Aldrich, PN 69794)
- 100% Isopropanol, ultrapure (e.g. AmericanBio, PN AB07015)

Procedure Workflow Overview



Mineral Oil Stabilized FFPE Sample Input Requirements and Guidelines

CAUTION: Do NOT go outside of the input requirements in the tables below. Doing so will negatively impact the quality and quantity of extractable nucleic acids.

Inputs listed as per microTUBE-500. A minimum of 2.0 mg of tissue is recommended.

Slide Section Input Requirements:

| Slide Collection Method | Minimum Input | Maximum Input |
|---|--|--|
| Scalpel or razor blade to scrape material from slides | 15 μm of total sample thickness (e.g., 3 slides at 5 μm thick = 15 μm total thickness) Min Area (on each slide): 7.5 mm x 7.5 mm | 40 μm of total sample thickness (e.g., 8 slides at 5 μm thick = 40 μm total thickness) Max Area (on each slide): 15 mm x 15 mm |

Curls/Scrolls Input Requirements:

| FFPE Curls/Scrolls Thickness | Minimum Input | Maximum Input |
|------------------------------|---------------|---------------|
| 5 μm | 2 | 8 |
| 10 μm | 1 | 4 |
| 15 μm | 1 | 2 |

Curls/Scrolls Input Requirements:

| FFPE Core Punch Diameter | Minimum Input | Maximum Input |
|------------------------------|---------------|---------------|
| \leq 1.2 mm; Length = 5 mm | 1 | 2 |

Sample Storage in Mineral Oil

1. Load each FFPE sample into a microTUBE-500.
2. Add 400 μl of mineral oil into each microTUBE-500. Ensure that the FFPE sample is fully immersed in the tube to prevent the sample from getting stuck in the Screw-Cap thread.
3. Close the microTUBE-500s tightly with their Screw-Caps.

NOTE: Samples can now be stored and shipped until extraction.

Removal of Mineral Oil

Mineral oil removal should be done just before commencing with the truXTRAC FFPE workflow to prevent oxidation-based aging of the bare tissue samples. Before beginning, follow Sections 2 through 4 in the main protocol.

1. Preheat dry block heaters to 56 $^{\circ}\text{C}$ (Step 13) and 80 $^{\circ}\text{C}$ (Step 3) \pm 3 $^{\circ}\text{C}$ as explained in Section 3.
2. Place the microTUBE-500s into the microTUBE-500 Centrifuge and Heat Block adapters.
3. Incubate the microTUBE-500s at 80 $^{\circ}\text{C}$ for 5 minutes.
4. Vortex briefly for 3 seconds.

5. Place the microTUBE-500s in the microTUBE-500 adapters. Then, with the bar code on the Screw-Cap sleeve facing outward, transfer microTUBE-500s into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 10 minutes.
6. Remove microTUBE-500s from microcentrifuge, remove Screw-Cap, and carefully remove all of the supernatant with a 200 µl pipette tip.

CAUTION: When removing the supernatant in the microTUBE-500, it is critical to not disturb the tissue. Depending on the tissue collection and input amount, the tissue could be a small pellet or large pieces of tissue.

- a. Locate the DNA-containing tissue pellet. It will be located on the same side as the barcode which faces outward during centrifugation.
- b. Slightly tilt the tube away from the tissue.
- c. Using a 200 µl pipette tip, slowly 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 PIPETTE TIPS.**

7. Add 400 µl of 100% isopropanol.
8. Vortex briefly for 3 seconds.
9. Place the microTUBE-500s in the microTUBE-500 adapters. Then, with the bar code on the Screw-Cap sleeve facing outward, transfer microTUBE-500s into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 10 minutes.
10. Remove microTUBE-500s from microcentrifuge, remove Screw-Cap, and carefully remove all of the supernatant with a 200 µl pipette tip.
11. Use a 20 µl pipette to remove the remaining liquid from the bottom without disturbing the tissue.
12. Leave the microTUBE-500s Screw-Caps off.
13. Place the opened microTUBES into a 56 °C heat block with the Screw-Caps removed for 10 minutes to evaporate the isopropanol.
14. Proceed with Section-5 (Paraffin Emulsification, Tissue Rehydration, & Lysis).

Appendix C - Troubleshooting Guide

| Issue | Cause | Solution | Comments / Suggestions |
|------------------------------|--|---|---|
| Low DNA yield | 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 and WB3, 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. |
| 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. |
| Bead clumping during elution | Residual paraffin in elution | Lower the amount of paraffin in the sample by trimming paraffin off the FFPE tissue block. | Too much paraffin in the input samples may not completely be removed during the purification. |

Tips for Determining Quality and Quantity of the Purified FFPE DNA

- To determine DNA 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. Kresse et al. (2018). Evaluation of commercial DNA and RNA extraction methods for high-throughput sequencing of FFPE samples. PLoS ONE 13(5): e0197456.
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.