truXTRAC® FFPE
DNA Plus Kit – Magnetic Beads (25)

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

PN 520262

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INTENDED USE

The truXTRAC FFPE DNA Plus Kit – Magnetic Beads (PN 520262) 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 incredibly simple in that it does not require any centrifugation steps.

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).

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].

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
010466	A	8/2018	Initial Release of truXTRAC FFPE DNA Plus Kit – Magnetic Beads
010466	B	10/2018	Format modification and layout improvement

KIT CONTENTS

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

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

STORAGE

Upon kit arrival, store the PK Solution and the Magnetic Bead Suspension at 2 to 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 microcentrifuge tubes or temperature-controlled water bath able to accurately heat between 50 to 90C
- Magnet Stand for 2 ml microcentrifuge tubes (e.g. Thermo Fisher Scientific, DynaMag™-2 Magnet, PN 12321D)

Required Chemicals

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

Optional Enzyme

- Optional DNase-free RNase A (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)

Optional Supplies for FFPE Sample Collection from Slides

- FFPE sectionPICK (Covaris, PN 520149)
- FFPE tissuePICK (Covaris, PN 520163)
- tissuePICK Forceps (5) (Covaris, PN 520164)
- 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>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>
Required Accessories (PN)	Insert XTU (500471)	ME220 Waveguide 4 Place (500534)	NA	Intensifier (500141)	Intensifier (500141)	NA

* 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

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

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-500
Scalpel or razor blade to scrape material from slides	**30 µm of total thickness** **Area: 10 mm²** **(6 slides at 5 µm thick = 30 µm total thickness)**

FFPE tissuePICK

Slide Collection Method	Maximum Input per microTUBE-500	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-500	Maximum FFPE Tissue Area per sectionPICK
sectionPICK PN 520149	2 sections from a sectionPICK	Max Area: 100 mm^2 Thickness range: 4 to 20 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.

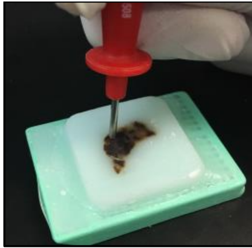
FFPE Curls/Scrolls Thickness	Maximum Scrolls per microTUBE-500
5 µm	4
10 µm	2
≤25 µm	1

FFPE core input requirements:

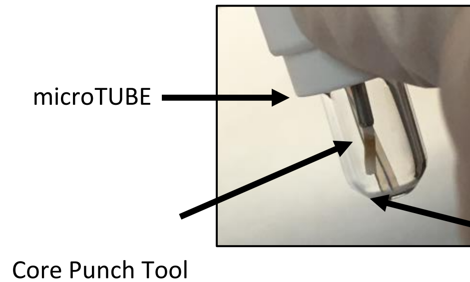
FFPE Core Punch Outer Diameter	Maximum Core Punches per microTUBE-500
≤ 1.2 mm (15 Gauge, outer) Length = 5mm	2

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

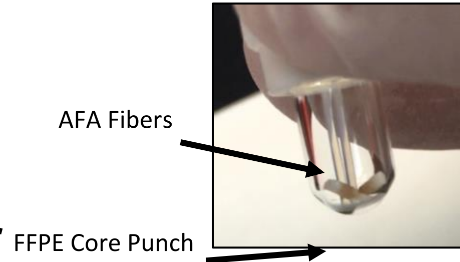
Core Punch Taken from Block



Loading Core Punch into microTUBE



Core Punches Loaded into microTUBE



2 – Preparation of Reagents

Follow these instructions before starting the FFPE DNA isolation protocol.

1. **DNA 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 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 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.

3 – Preparation of Heat Blocks

1. Preheat dry block heaters or water baths to 56C and 80C ± 2C. 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 heat block adaptor and a microTUBE-500 filled with water into the heat block.
   b. Immerse a thermometer into the microTUBE-500.
   c. Wait until the temperature has reached the plateau.

d. Adjust the Set-temperature accordingly until the temperature inside the microTUBE-500 has reached 56C or 80C ± 2C.

CAUTION: The Covaris microTUBE-500 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

Create an "*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.

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

Instrument	M220	ME220	S220	E220 Evolution	E220	LE220
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)	NA	X Dither: 1 mm Z Dither: 2 mm Dither Speed: 10 mm/sec	NA	NA	NA	X Dither: 1.5 mm Z Dither: 3 mm Dither Speed: 10mm/sec
Water Level (run)	Full	Auto	8	6	6	5

5 – Paraffin Emulsification, Tissue Rehydration, and Lysis

1. Prepare the heat blocks according to Section 3.

2. Prepare the DNA 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 DNA Lysis Buffer/Proteinase K Mix should be kept at room temperature and used within 30 min after preparation.

Table 1 – DNA Lysis Buffer/Proteinase K Mix

Reagent	Volume for one sample*	Volume for N samples*
DNA Lysis Buffer	**396** µl	**396** µl x N
Proteinase K Solution	**88** µl	**88** µl x N

* calculation includes 10% excess in final volume

3. Open the microTUBE-500 Screw-Cap and load the FFPE tissue.
4. Add **440** µl of the DNA Lysis Buffer/Proteinase K mix to the microTUBE-500. FFPE tissue samples may also be added directly to microTUBE-500s containing the DNA Lysis Buffer/Proteinase K mix.

Note: If adding FFPE tissue into the microTUBE-500s containing the DNA Lysis Buffer/Proteinase K mix, ensure that the FFPE sample is fully immersed to prevent the sample from getting stuck in the Screw-Cap thread.

5. 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-500s into the Focused-ultrasonicator for processing.
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. See example below.

Before AFA

After AFA

7. Remove the microTUBE-500 from the Focused-ultrasonicator and load it into the microTUBE-500 Centrifuge and Heat Block adapter on the heat block set to 56C.
8. Incubate for 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.

9. 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.
10. Incubate for 75 minutes at 80C.
11. Remove microTUBE-500 with adapter from the heat block and let cool for 3 minutes at room temperature.
12. 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 Section 6 for DNA purification.

6 – DNA PURIFICATION

1. Verify that the heat block is set to 56C (Steps 5 and 24).
2. Prepare the BB3/Magnetic Bead Mix according to the Table 2.

Table 2 – 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.

3. Add **728** µl of the BB3/Magnetic Bead Mix to the DNA solution in the 2 ml microcentrifuge tube.
4. Cap the 2 ml microcentrifuge tube and vortex for 10 seconds.
5. Incubate the 2 ml microcentrifuge tube at 56C for 5 minutes.
6. Place the 2 ml microcentrifuge 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.

7. With the 2 ml microcentrifuge tube still on the magnet, carefully remove and discard the supernatant. Avoid touching or disturbing the bead pellet.
8. Add **1** ml Buffer WB3 to the tube.
9. 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.
10. Place the 2 ml microcentrifuge tube back on the magnet stand and wait for 5 minutes until the beads have been pulled to the side by the magnet.
11. With the 2 ml microcentrifuge tube on the magnet, carefully remove and discard the supernatant.
12. Repeat wash steps 8 through 11. 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.

13. Add **1** ml 80% ethanol into the 2 ml microcentrifuge tube.
14. 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.
15. Place the tube on the magnetic stand and wait for 2 minutes until beads have been pulled to the magnet.
16. With the tube on the magnet, carefully remove and discard the supernatant.
17. Remove the tube from the magnetic stand and add **300** µl 80% ethanol.
18. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended.
19. Place the tube on the magnetic stand and wait for 2 minutes until the beads have been pulled to the magnet.
20. With the tube on the magnet, carefully remove and discard as much supernatant as possible. Use a 20 µl pipet to remove the remaining liquid from the bottom of the tube.
21. Leave the tube open on the magnetic stand and let the beads dry for 8 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.

DNA Elution

22. Remove the tube from the magnetic stand and add **50** to **100** µl of elution 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.

23. Resuspend the beads by pipetting up and down 20 times. Ensure that all beads are submerged in the buffer and are fully resuspended.
24. Cap the tube and incubate the 2 ml microcentrifuge tube in the heat block set to 56C for 5 minutes.
25. Remove the tube from the heat block and place it on the magnetic stand and wait for 2 minutes.
26. 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.

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

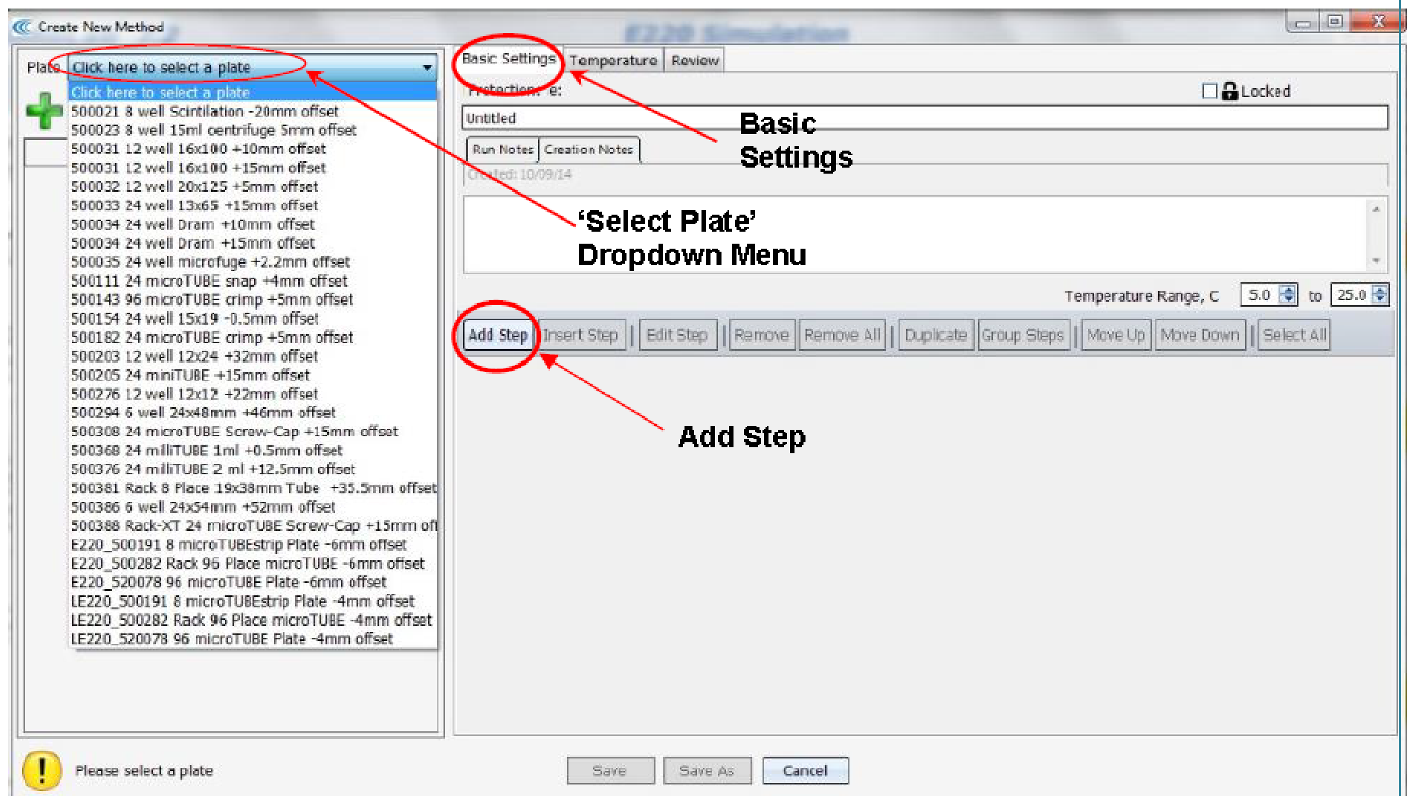
APPENDIX A – PROTOCOL CREATION IN SONOLAB

LE220 with SonoLab 7.3

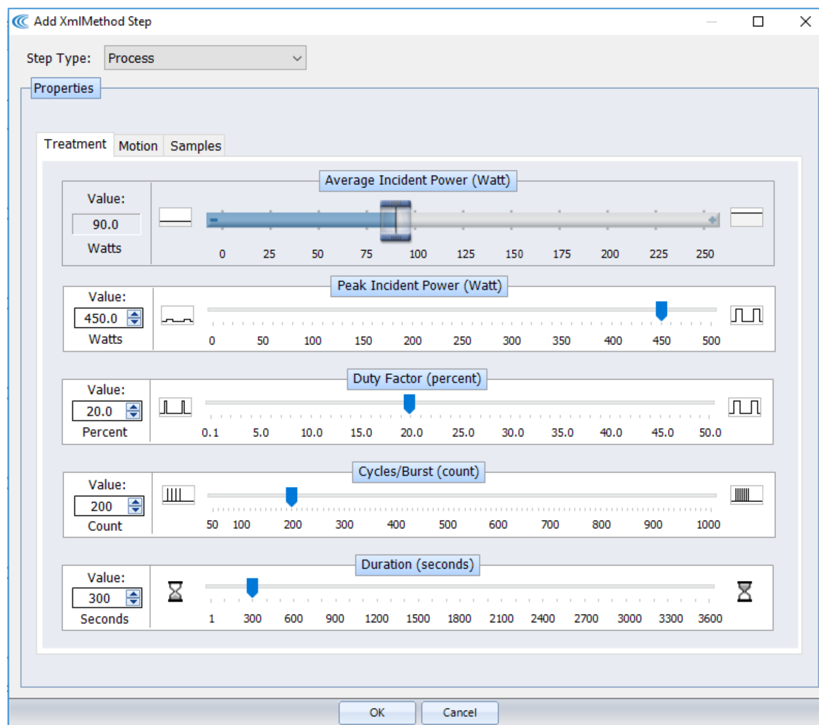
Refer to the E/LE Series Manual https://covaris.com/wp-content/uploads/010277-E-and-LE-series-Manual_Rev-F.pdf

1. On the Run screen, click the "New…" button in the Method window.
2. In the Basic Settings tab, first select the 500452 plate with z dither enabled.

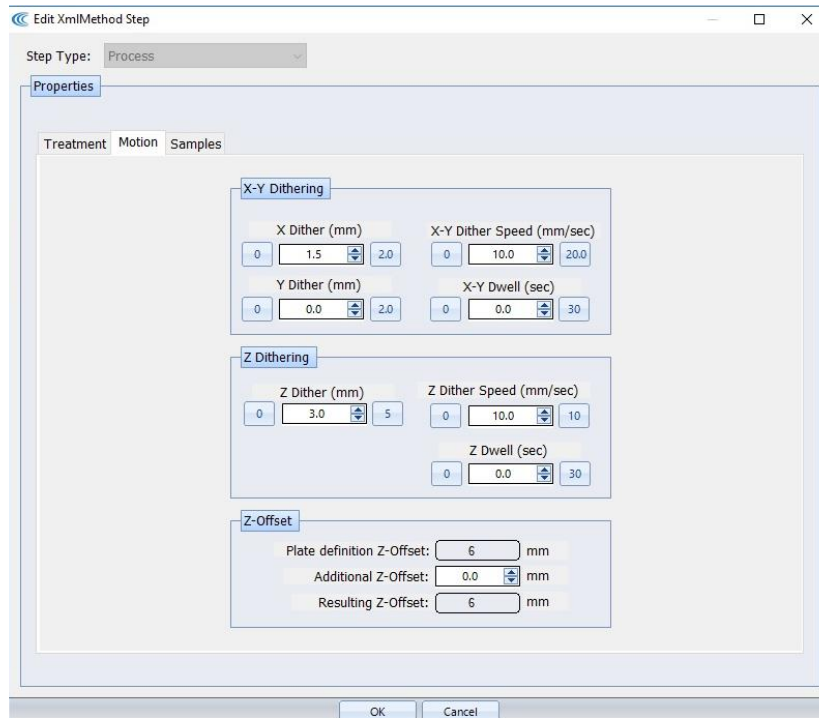
Plate: Plate 500452-24 microTUBE-500 Screw-Cap +6mm offset z enable



3. Rename the "Untitled" protocol to "***Acoustic Paraffin Emulsification***".
4. Click the "Add Step" button. In the Treatment tab, input the following AFA settings shown below.



5. In the Motion tab, input the X- and Z- dithering values shown below.

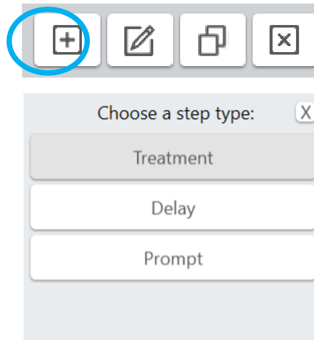


6. Click the “OK” button.
7. Select the sample rows in the Plate window to run.
8. Click the “Save” button.

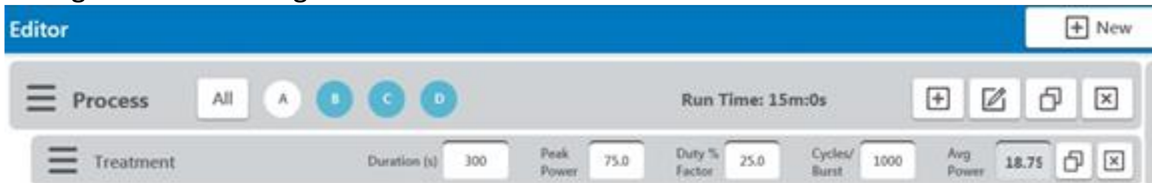
ME220 with Sonolab 8

Refer to the ME220 User Manual. https://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".

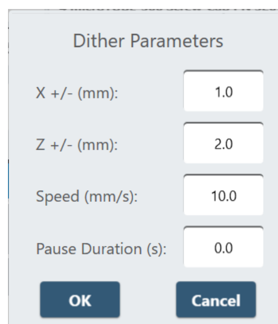


6. Change the AFA settings in the Treatment bar as shown below.



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.

APPENDIX B – 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(s) 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^{TM} (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 [2].

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.