truXTRAC® FFPE DNA microTUBE Kit – Column Purification (25)

(PN 520136)

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Intended Use

The truXTRAC FFPE DNA Kit is intended for use in molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Introduction

The truXTRAC FFPE DNA Kit is designed for the controlled and efficient extraction of DNA from Formalin Fixed, Paraffin Embedded (FFPE) tissue samples with the Covaris Adaptive Focused Acoustics® (AFA). AFA enables the active removal of paraffin from FFPE tissue samples in aqueous buffer, allowing simultaneous tissue rehydration. Compared to traditional passive, chemical-based methods of paraffin removal, this mechanical process is not as limited by the thickness of FFPE tissue sections. The AFA process enables the use of thicker sections, which can increase DNA yield and minimize the impact of increased DNA degradation at the exposed surfaces of a section. The truXTRAC process results in high yields of high-quality DNA well suited for analytical methods such as next-generation sequencing or qPCR.

This protocol is optimized for sections up to 25 µm in thickness and cores up to 1.2 mm in diameter.

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 sections, the ratio of tissue to wax, the type of tissue, and the age of the FFPE block are the main causes for this variability.

The quality of DNA isolated from FFPE samples is also highly variable. During the fixation process, DNA is cross-linked to proteins and other nucleic acid molecules to varying degrees. Incomplete reversal of this crosslinking may cause the isolated DNA to perform less well in downstream applications such as PCR and qPCR. In addition, the size of DNA fragments isolated from FFPE samples is generally smaller than that of DNA isolated from fresh or frozen tissues. This is particularly evident in older FFPE sample blocks or sample blocks stored at elevated temperatures.

Note for First Time Users:

Given the highly variable yield of DNA from FFPE tissue blocks, we recommend using FFPE blocks that have been well characterized for yield and quality for initial testing of the truXTRAC FFPE kit. Ideally, samples should be extracted immediately after sectioning. Please contact Covaris at Application Support (applicationsupport@covaris.com) if you have any questions.

Kit Contents

- Tissue SDS Buffer ..... 10 mL
- B1 Buffer ..... 7.5 mL
- B5 Buffer ..... 7 mL
- BW Buffer ..... 15 mL
- BE Buffer ..... 7.5 mL
- PB Buffer ..... 1.5 mL
- Proteinase K ..... 30 mg
- Purification Columns 25
- Collection Tubes 50
- microTUBE-130 AFA Fiber Pre-Slit Screw-Cap .. 25

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

Storage

This kit should be stored at room temperature (18 – 25 °C) upon receipt. After resuspension, Proteinase K should be stored at -20 °C.

Laboratory Equipment, Chemicals, and Consumables Supplied by User

Required Products per Instrument

Instrument	M220	ME220	S220*	E220*evolution*	E220*	L-Series
Rack/Holder	Holder XTU (PN 500414 & Insert XTU (PN 500489)**	Rack 4-place micro-TUBE Screw-Cap (PN 500522)	Holder microTUBE Screw-Cap (PN 500339)	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack 24 Place micro-TUBE Screw-Cap (PN 500308)	Rack 24 Place micro-TUBE Screw-Cap (PN 500308)
Instrument Specific Requirements	N/A	Waveguide 4 Place (PN 500534)	N/A	Intensifier (PN 500141)	Intensifier (PN 500141)	N/A
Accessories	Centrifuge and Heat Block microTUBE Adapter (PN 500406)					

*The E210 and S210 instruments have been obsoleted.
**Holder PN 500358, although discontinued, can be used. This holder does not require an insert.

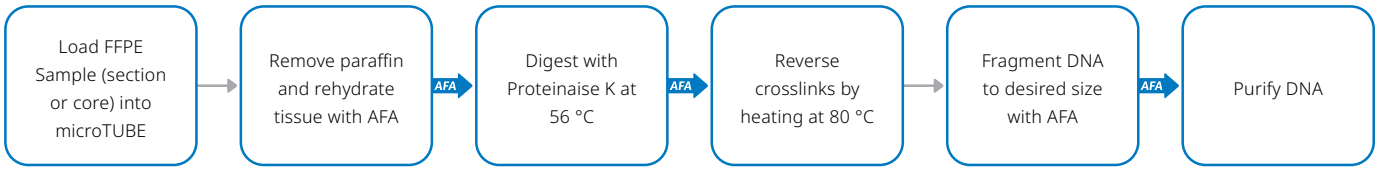
Other Supplies

- Microcentrifuge with 11,000 x g capability
- Water bath, oven, or dry block heater (e.g., Eppendorf ThermoMixer) for 1.5 or 2 mL tubes, capable of heating to 80 °C
- RNase A, DNase and protease-free (10mg/mL) (e.g., Thermo Scientific PN EN0531)
- Ethanol (> 96%), molecular-biology grade (e.g., Thermo Scientific PN BP2818-100)
- 1.5 mL nonstick nuclease-free microcentrifuge tubes (e.g., Life Technologies PN AM12450)

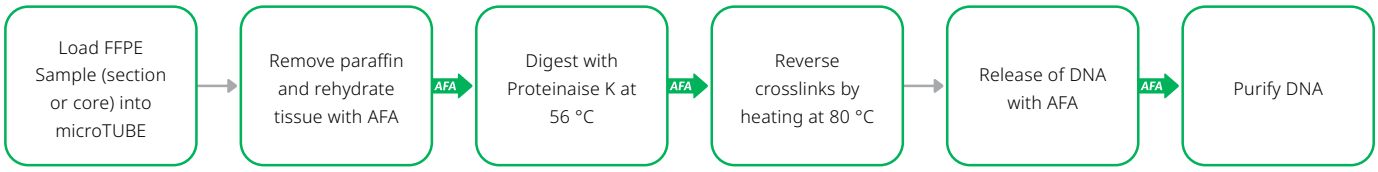
Procedure Workflow Overview

Three different options are possible with the truXTRAC FFPE DNA Kit. The three options differ in the workflows for DNA extraction.

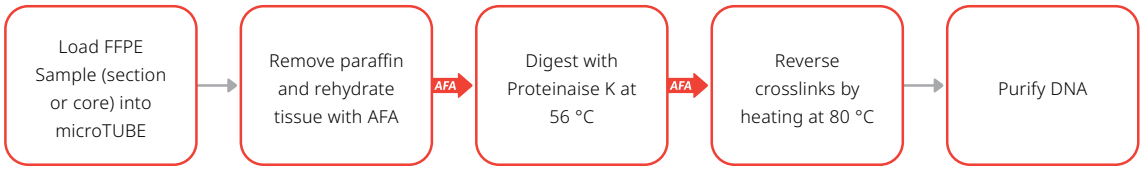
- **Option A: Extract and Fragment DNA (for NGS)** - Shear DNA during extraction to a size suitable for next-generation sequencing library construction. Fragment size can be tuned between 200 and 400 bp.



- **Option B: Extract Large DNA Fragments (>2 kb)* with Improved Yield** - Extract ~2 kb DNA fragments. This protocol is recommended for most analytical applications, including PCR. Note that actual DNA fragment size will depend of the quality of the starting material.



- **Option C: Extract Genomic DNA*** - Extract large "genomic" DNA without any additional fragmentation. Actual DNA fragment size will depend on the quality of the starting material. For high-quality FFPE tissue blocks, we typically see an average fragment size of >= 8 kb.



Only Option C is described in the main part of the protocol, please refer to ***Appendix A*** for Options A and B. Please refer to ***Appendix B*** for examples of final DNA fragment size distribution.

*Actual DNA fragment size will depend of the quality of the starting tissue block.

FFPE Tissue Sample

1. Sample Input Requirements

The truXTRAC process is highly efficient at removing paraffin even from relatively thick FFPE sections while simultaneously rehydrating the tissue. Use of thicker sections is often desirable, both for increased yield and that DNA or RNA in the exposed surfaces of a section tends to degrade quickly. We recommend using sections between 15 and 25 µm thick, or cores of 1.2 mm.

NOTE: Excess paraffin will adversely affect the yield and quality of DNA extracted from FFPE. We strongly advise trimming off any excess of paraffin before sectioning an FFPE tissue block, or after the section has been cut from the FFPE block. A ratio of 80% tissue to 20% paraffin or higher is ideal.

	FFPE Sections Mounted on Slide	FFPE Sections "Scrolls" or "Curls"		FFPE Cores
Size (thickness or diameter)	4 to 10 µm	7 to 15 µm	16 to 25 µm	< 1.2 mm diameter
Size (length)	N/A	< 10 mm**		
Collection Tool	Scalpel or razor blade	Tweezers		May be loaded directly from core punch or transferred using tweezers or forceps.
Maximum Number of Samples Per Tube*	2 (200 mm^2 tissue for a 5 µm section)	2*	1*	1*

*Numbers represent trimmed sections only. **If the FFPE sample is longer than about 10 mm, cut it in half before loading.

CAUTION: The total mass of FFPE sample processed per extraction should be between 2 to 5 mg. Lower amounts may result in insufficient yield and higher amounts may cause spin columns to become partially or fully clogged.

2. Tissue Fixation Requirements

The yield and quality of DNA extracted from FFPE tissue blocks is highly dependent on tissue collection and paraffin embedding procedures. For good yields of high quality DNA:

- Use a maximum fixation time of 24 hours
- Use Formalin solution, neutral buffered, 4%
- Fix tissue sample as quickly as possible after collection

Buffers

1. Add ethanol to Buffer B5: Add 28 mL of ethanol (> 96%) to Buffer B5 concentrate and mark label on the cap. After preparation, Buffer B5 can then be stored for one year at room temperature.
2. Resuspend Proteinase K: Add 1.35 mL Buffer PB to the lyophilized Proteinase K vial. Proteinase K solution can be stored for 6 months at -20 °C. When re-suspended in the supplied Buffer PB, the solution should not freeze when stored at -20 °C.
3. Check Buffer B1 and Tissue SDS Buffer: A white precipitate may form during storage. Incubate the bottles at 50 - 70 °C for 5 to 10 minutes before use to dissolve any precipitate.

Focused-ultrasonicator

For more detailed instructions on how to prepare your particular instrument please refer to your instrument's User Manual.

- S, E, or LE-Series Focused-ultrasonicators, set up the instrument as shown in Table below. Wait for the water to reach temperature and to degas.
- M220 Focused-ultrasonicators, put the Holder (PN 500414) and the Insert (PN 500489 or the discontinued Holder PN 500358 without insert) in place and fill the water bath until the water reaches the top of the holder. Allow system to reach temperature (20 °C).
- ME220 Focused-ultrasonicators, position the ME220 Waveguide 4 Place (PN 500534) into place in the water bath. Allow system to reach 20 °C. Load samples into Rack 4 Place (PN 500522) and place into the rack holder.

Instrument	Water Level (RUN)	Temperature (° C)	Plate Definition***
M220*	16 mL*	20	N/A
S220	15	18	N/A
ME220**	Automatic**	20	"4 microTUBE-130 Screw-Cap PN 520216.2rck"
E220	10	18	"E220_500308 Rack 24 Place microTUBE-130 Screw-Cap +15mm offset"
E220evolution	10	18	"500432 E220e 4 microTUBE-130 Screw Cap 0.18mm offset"
L-Series	15	18	"LE220_500308 Rack 24 Place microTUBE-130 Screw-Cap +15mm offset" "LE220PRSC_500308 Rack 24 Place microTUBE-130 Screw-Cap +15mm offset"

*The M220 requires a fill volume of ~16 mL using the included wash bottle for filling. Ensure that the water is at the top of the tube insert before AFA processing.
**The ME220 has an attached Water Works System that automatically fills the water bath to the level appropriate for the consumable that is linked to the method selected.
*** If the instrument does not have the desired plate definition file, please contact Covaris Technical Support at techsupport@covaris.com

Heating Blocks, Water Baths, or Ovens

Preheat dry block heaters, water baths, or ovens to 56 °C (or T set1 - see ***Appendix E***) and 80 °C (or T set2 - see ***Appendix E***).

> **NOTE:** When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters (PN 500406) first.

It is important to confirm that the desired temperatures are actually reached. See ***Appendix E*** for instructions on how to calibrate your heating device. Subsequent steps will require 70 °C.

DNA Extraction from FFPE Tissue: Deparaffinization, Protein Digesting, & Decrosslinking

Option C: Extract Genomic DNA

Option C is designed for extraction of the largest possible DNA fragments from FFPE tissue. Note that actual DNA fragment size will depend of the quality of the starting material.

1. Open microTUBE Screw-Cap, add 100 µL Tissue SDS Buffer into microTUBE, and load FFPE tissue (section or core). Affix Screw-Cap back in place.

> **NOTE:** If the FFPE tissue samples are loose or broken the samples may be added to the microTUBE prior to Tissue SDS Buffer addition to facilitate easier loading.

2. Process the sample using the settings provided in ***Table 1*** to dissociate the paraffin and rehydrate the tissue. During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified (see ***Appendix C***).

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	300	20
S2 or E210*	5 (intensity)	10	200	300	20
M220	75	20	200	300	20
ME220	75	25	1,000	390	20
E220evolution	175	10	200	300	20
LE220	450**	30	200	300	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 1. Paraffin removal and tissue rehydration settings.

3. Open microTUBE Screw-Cap, add 20 µL of Proteinase K solution to the sample, and affix Screw-Cap back in place.
4. Process the sample using the settings provided in ***Table 2*** to properly mix Proteinase K with the sample.

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	10	20
S2 or E210*	5 (intensity)	10	200	10	20
M220	75	20	200	10	20
ME220	75	25	1,000	10	20
E220evolution	175	10	200	10	20
LE220	450**	30	200	10	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 2. Proteinase K mixing settings.

5. Proteinase K digestion at 56 °C (or T set$_1$ - see ***Appendix D***): Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56 °C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µL of Proteinase K solution, mix, and incubate for 1 more hour. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.
6. Crosslink reversal at 80 °C (or T set$_2$ - see ***Appendix D***): Incubate samples for 1 hour at 80 °C to reverse formaldehyde crosslinks. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.

> **NOTE:** If the same heating source is being used for both the 56 °C & 80 °C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80°C or T set$_2$.

7. Proceed to ***Section 3*** – DNA Purification.

DNA Purification

NOTE: Set heating source to 70 °C and preheat the required volume of Buffer BE in a 1.5 mL microcentrifuge tube: (number of samples x 100 µL x 1.1).

1. Transfer the sample to a clean 1.5 mL microcentrifuge tube.
   Optional: The sample can be treated with RNase A to remove RNA before DNA purification. Add 5 µL of RNase A solution and incubate for 5 minutes at room temperature.
2. Add 140 µL Buffer B1 to your sample and vortex thoroughly.
3. Add 160 µL ethanol (> 96%) to the sample and vortex thoroughly.
4. Centrifuge at 10,000 x g for 2 minutes at room temperature. After centrifugation, much of the paraffin will have formed a white layer, floating on top of the liquid.
5. Place a Purification Column into a provided Collection Tube.
6. While holding the sample tube at about the same angle as in the rotor, use a pipette to slowly recover the liquid layer, and transfer to the column. Transfer of a small amount of paraffin particles to the column is acceptable and will not interfere with the DNA purification.
7. Spin the assembly at 11,000 x g for 1 minute.
8. Discard the flow-through and place the column back in the Collection Tube.
9. ***1st wash***: Add 500 µ.L Buffer BW. Spin the assembly at 11,000 x g for 1 minute.
10. Discard the flow-through and place the column back in the Collection Tube.
11. ***2nd wash***: Add 600 µL Buffer B5. Spin the assembly at 11,000 x g for 1 minute.
12. Discard the flow-through and place the column in a new Collection Tube (provided).
13. ***Dry column***: Spin the assembly at 11,000 x g for 1 minute.
14. ***Elute DNA - 1st step***: Place the Purification Column into a new 1.5 mL microfuge tube (not provided) and add 50 µL pre-warmed Buffer BE (70 °C) to the center of the column. Incubate at room temperature for 3 minutes. Spin the assembly at 11,000 x g for 1 minute.
15. ***Elute DNA – 2nd step***: Add a second aliquot of 50 µl pre-warmed Buffer BE. Incubate again at room temperature for 3 minutes. Spin the assembly at 11,000 x g for 1 minute.
16. ***DNA is eluted in 100 µL Buffer BE*** (5 mM Tris HCl pH 8.5).

NOTE: To increase the concentration of the recovered DNA, perform the second elution with the eluate from the first elution: Reload the first eluate to the top of the column, incubate for 3 minutes at room temperature and re-spin the assembly at 11,000 x g for 1 minute collecting the sample in the same 1.5 mL microcentrifuge tube. The eluted DNA is now in 50 µL.

Appendix A: Alternative Protocols for DNA Extraction from FFPE

Note for ME220 Users: AFA Settings for the ME220 instrument have yet to be determined for Option A or Option B.

Option A: Extract and Fragment DNA (for NGS)

This protocol allows direct fragmentation of DNA to a size suitable for Next Generation Sequencing library construction during the extraction process.

1. Open microTUBE Screw-Cap, add 100 µl Tissue SDS Buffer into microTUBE, and load FFPE tissue (section or core). Affix Screw-Cap back in place.

> **NOTE:** If the FFPE tissue samples are loose or broken the samples may be added to the microTUBE prior to Tissue SDS Buffer addition to facilitate easier loading.

2. Process the sample using the settings provided in ***Table 3*** to dissociate the paraffin and rehydrate the tissue. During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified (see ***Appendix C***).

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	300	20
S2 or E210*	5 (intensity)	10	200	300	20
M220	75	20	200	300	20
E220evolution	175	10	200	300	20
LE220	450**	30	200	300	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 3. Paraffin removal and tissue rehydration settings.

3. Open microTUBE Screw-Cap, add 20 µL of Proteinase K solution to the sample, and affix Screw-Cap back in place.
4. Process the sample using the settings provided in ***Table 4*** to properly mix Proteinase K with the sample.

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	10	20
S2 or E210*	5 (intensity)	10	200	10	20
M220	75	20	200	10	20
E220evolution	175	10	200	10	20
LE220	450**	30	200	10	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 4. Proteinase K mixing settings.

5. Proteinase K digestion at 56 °C (or T set$_1$ - see ***Appendix E***): Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56 °C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µL of Proteinase K solution, mix, and incubate for 1 more hour. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.
6. Crosslink reversal at 80 °C (or T set$_2$ - see ***Appendix E***): Incubate samples for 1 hour at 80 °C to reverse formaldehyde crosslinks. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.

> **NOTE:** If the same heating source is being used for both the 56 °C & 80 °C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80 °C or T set$_2$.

7. DNA fragment size can be tuned to the desired average fragment size by following the listed instrument specific settings below.

> **NOTE:** If the target size is not achieved then the treatment time should be adjusted.

E- and S-Series Focused-ultrasonicator

Targeted Fragment Size (bp)	Peak Incident Power (W) (S220, E220, & E220*evolution*)	Intensity (S2 and E210)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
200	175	5	10	200	300	20
300	175	5	10	200	110	20
400	175	5	10	200	80	20

M220 Focused-ultrasonicator

Targeted Fragment Size (bp)	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
200	75	20	200	450	20
300	75	20	200	200	20
400	75	20	200	120	20

LE220 Focused-ultrasonicator

Targeted Fragment Size (bp)	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
200	450*	30	200	300	20
300	450*	30	200	150	20
400	450*	30	200	80	20
*As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.					

8. Proceed to ***Section 3*** – DNA Purification.

Option B: Extract Large DNA Fragments (>2 kb) with Improved Yield

Option B enhances the release of DNA from the tissue while preserving a fragment size of > 2 kb. Note that actual DNA fragment size will depend of the quality of the starting material.

1. Open microTUBE Screw-Cap, add 100 µL Tissue SDS Buffer into microTUBE, and load FFPE tissue (section or core). Affix Screw-Cap back in place.

> **NOTE:** If the FFPE tissue samples are loose or broken the samples may be added to the microTUBE prior to Tissue SDS Buffer addition to facilitate easier loading.

2. Process the sample using the settings provided in ***Table 5*** to dissociate the paraffin and rehydrate the tissue. During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified (see ***Appendix C***).

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	300	20
S2 or E210*	5 (intensity)	10	200	300	20
M220	75	20	200	300	20
E220evolution	175	10	200	300	20
LE220	450**	30	200	300	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 5 Paraffin removal and tissue rehydration settings.

3. Open microTUBE Screw-Cap, add 20 µL of Proteinase K solution to the sample, and affix Screw-Cap back in place.
4. Process the sample using the settings provided in ***Table 6*** to properly mix Proteinase K with the sample.

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	175	10	200	10	20
S2 or E210*	5 (intensity)	10	200	10	20
M220	75	20	200	10	20
E220evolution	175	10	200	10	20
LE220	450**	30	200	10	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 6. Proteinase K mixing settings.

5. Proteinase K digestion at 56 °C (or T set$_1$ - see ***Appendix E***): Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56 °C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µL of Proteinase K solution, mix, and incubate for 1 more hour. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.
6. Crosslink reversal at 80 °C (or T set$_2$ - see ***Appendix E***): Incubate samples for 1 hour at 80 °C to reverse formaldehyde crosslinks. When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.

> **NOTE:** If the same heating source is being used for both the 56 °C & 80 °C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80 °C or T set$_2$.

7. DNA fragment size can be tuned to the desired average fragment size by using the settings in ***Table 7*** below.

Instrument	Peak Incident Power (W)	Duty Factor (%)	Cycles per Burst (#)	Treatment Time (sec)	Temperature (°C)
S220 or E220	105	10	200	10	20
S2 or E210*	3 (intensity)	10	200	10	20
M220	75	20	200	10	20
E220evolution	105	10	200	10	20
LE220	300**	30	200	10	20

*These instruments have been obsoleted.
**As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

Table 7. DNA release with AFA.

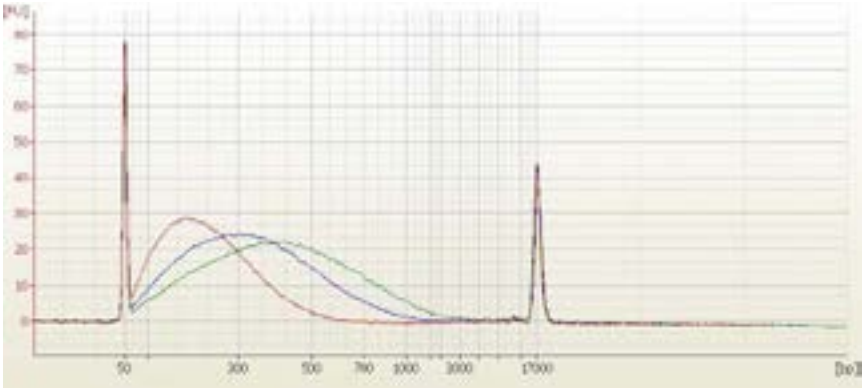
8. Proceed to ***Section 3*** – DNA Purification.

Appendix B: Examples of DNA Fragment Size Distribution

In these examples, DNA has been extracted from 10 µm sections from the same kidney tissue block with the Covaris FFPE kit following the 3 available options. The Bioanalyzer electropherograms below represent the fragment size distribution after purification. The size of the non-fragmented, genomic DNA (Option C) depends of the previous storage condition of the tissue block, including how it has been fixed and paraffin embedded.

200, 300 and 400 bp Peaks

Option A: Extract and fragment DNA (for NGS) Subsequently to extraction, DNA is sheared to a size suitable for Next Gen Sequencing (NGS) library construction. In this example, one sample has been sheared to 200 bp, one to 300 bp and one to 400 bp.



3 kb Peak

Option B: Extract large DNA fragments (> 2 kb)*

AFA energy is used to release the DNA from the tissue. During this process the DNA is also sheared into fragments larger than 2 kb.

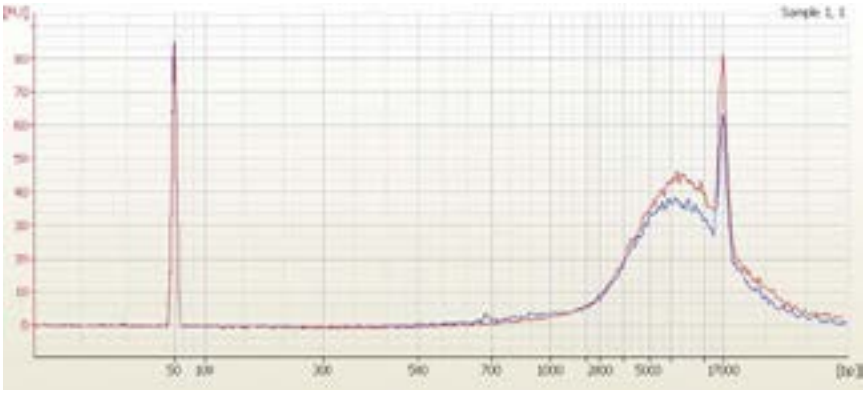
*Final size will depend of the quality of the starting tissue.

8 kb Peak

Option C: Extract genomic DNA*

DNA size will be the largest possible and will depend on the quality of the starting tissue.

*Final size will depend of the quality of the starting tissue.



Appendix C: Paraffin Emulsification with AFA Energy

Paraffin is emulsified in microTUBE Screw-Cap using a Covaris S220 Focused-ultrasonicator. Sample before (left side) and after (right side) processing. Sample was a 10 µm kidney tissue section.

Appendix D: Troubleshooting Guide

Issue	Possible Causes	Solution	Comments and Suggestions
Low yield of DNA	Low tissue to wax ratio in FFPE section	Repeat the procedure using additional sections until desired yield is achieved.	In the initial use of the truXTRAC FFPE kit, use FFPE blocks that have been well characterized for yield and quality.
	Insufficient tissue input	Increase FFPE tissue section thickness or use more sections up to 5 mg total weight.	N/A
	Proteinase K stored above recommended temperature or expired	Repeat the procedure using fresh Proteinase K.	Always store proteinase K solution at -20 °C.
No DNA	Ethanol not added to buffer B5	Repeat the procedure with fresh samples and ensure ethanol is added to buffer B5.	N/A
	Step 4 of option A, B or C has been omitted	Repeat the procedure	Step 4 mixes the Proteinase K with the sample. As PB Buffer contains Glycerol, it falls at the bottom of the microTUBE and won't be in contact with the sample without this mixing step
DNA concentration is too low	Elution volume is too high.	Repeat procedure using lower elution volume (50 µL minimum volume is required). Concentrate samples using ethanol precipitation or other means.	N/A
DNA does not perform well in downstream applications such as qPCR	DNA in FFPE sample blocks is severely cross-linked or degraded	Design amplicons to be as small as possible (< 100 bp).	DNA isolated using Covaris AFA technology is of the highest possible quality. Some FFPE sample blocks may be too degraded or cross-linked for some applications.
DNA fragments size too large when following Option A	Too much emulsified paraffin in the sample	Trim any excess paraffin from tissue blocks before proceeding with protocol. We recommend running a time course at step 7 and to increase the treatment time by 30 seconds steps.	Too much emulsified paraffin absorbs some of the acoustic energy and will adversely affect DNA Shearing efficiency.

Appendix E: Heating Source Calibration Procedure

1. If using a dry block heater, place the Covaris Heat Block microTUBE Adapters into the heating block.
2. Add water to one of the Heat Block microTUBE Adapters and insert a glass thermometer or place a glass thermometer into the water bath or the oven.
3. Set the heating source temperature to 56 °C.
4. Wait for the heating source to reach the set point.
5. Check temperature displayed by the thermometer (Tth).
6. If Tth is between 55 °C and 57 °C (setpoint +/- 1 °C), use 56 °C for $Tset_1$.
7. Otherwise, use this formula (T set_1 (˚C) = 120˚C- Tth) to obtain T set_1.
8. Repeat steps 3 - 7 with an initial set point of 80˚C and formula (T set_2 (˚C) = 160˚C- Tth) to obtain T set_2.

Appendix F: Removing or Installing the Intensifier from an E-Series Instrument (PN 500141)

The 500141 Intensifier is a small, inverted stainless-steel cone centered over the E-Series transducer by four stainless wires. The wires are held in place by a black plastic ring pressed into the transducer well.

If an AFA protocol requires “no Intensifier”, please remove the Intensifier, using the following steps:

1. Empty the water bath. Start the instrument and start the SonoLab software.
2. Wait for the homing sequence to complete (the transducer will be lowered with the rack holder at the home position, allowing easy access to the Intensifier).
3. Grasp opposite sides of plastic ring and gently pull the entire assembly out of the transducer well. Do not pull on the steel cone or the wires. The ring is a friction fit in the well – no hardware is used to hold it in place.

The 500141 Intensifier (left) shown installed in the E-Series transducer well and (right) removed. Note the “UP” marking at the center of the Intensifier.

If a protocol requires the Intensifier to be present, simply reverse this process:

4. Align the black plastic ring with the perimeter of the transducer well. Note that the flat side of the center cone (marked UP) should be facing up (away from the transducer).
5. Gently press each section of the ring into the well until the ring is seated uniformly in contact with the transducer, with approximately 2 mm of the ring evenly exposed above the transducer assembly. Do not press on the cone or wires. The rotation of the ring relative to the transducer assembly is not important.
6. Refill the tank. Degas and chill the water before proceeding.

Additional Notes

1. Covered by US Patent 9,080,167
2. Other patents pending
3. Best Practices for determining the yield and purity of isolated DNA:
    - To determine DNA yield with the highest level of accuracy, a fluorometric assay such as Qubit TM (Life Technologies) should be used.
    - In addition, spectrophotometric analysis of DNA for A260/280 and A260/230 ratios will determine if protein or peptide/salt contamination is present in the sample.
4. Tissue Blocks were obtained from: Theresa Kokkat, PhD and Diane McGarvey, Cooperative Human Tissue Network (CHTN), Eastern Division, University of Pennsylvania, USA
5. See following link: https://www.covaris.com/wp/wp-content/uploads/resources_pdf/pn_010178.pdf for updates to this document.
6. 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.

Technical Assistance

Technical Support – Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone
    - United States: Tel: +1 781.932.3959 during the hours of 9:00 a.m. to 4:00 p.m., Monday through Friday, Eastern Standard Time/EST (UTC–05:00)
    - Europe: Tel: 44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Greenwich Mean Time/GMT
- E-mail instrumentation queries to techsupport@covaris.com or application queries to applicationsupport@covaris.com

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