PROTOCOL

Covaris

truChIP® FFPE Chromatin Shearing Kit

Adaptive Focused Acoustics® (AFA®)-based chromatin shearing for ChIP-based applications from FFPE tissues

PN 520257

Contents

INTENDED USE

The truChIP FFPE Chromatin Shearing Kit is intended for use in research applications only (RUO). This product is not intended for the diagnosis, prevention, or treatment of disease.

INTRODUCTION

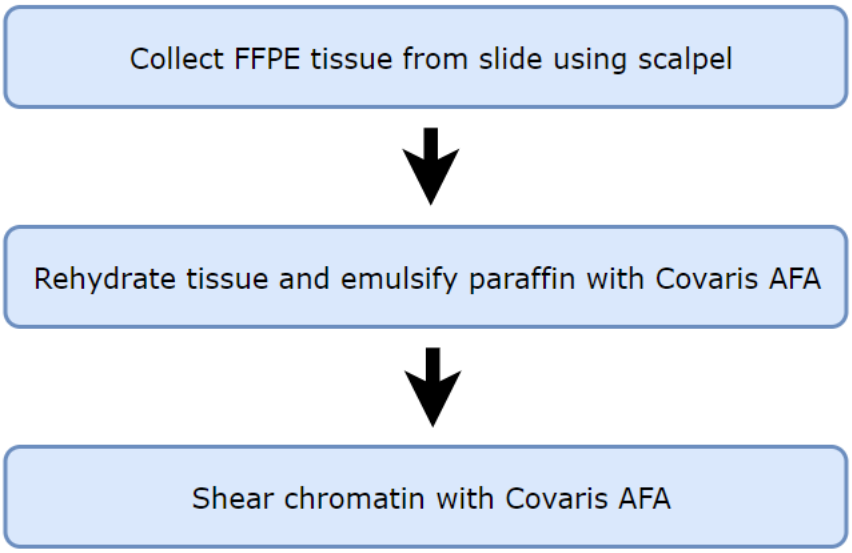
The truChIP FFPE Chromatin Shearing Kit utilizes an active-based paraffin emulsification process prior to shearing chromatin using Covaris AFA Focused-ultrasonicators. This protocol provides a fast, non-contact, isothermal, and reproducible shearing method for histone marks as well as transcription factor ChIP assays. Formalin-fixed, paraffin-embedded (FFPE) samples have not been routinely used in chromatin immunoprecipitation because of limited amounts of material, and reduced antigen availability due to the harsh fixation process. This truChIP FFPE Chromatin Shearing Kit can be used as an alternative to cumbersome methods that require multiple organic solvents and rehydration series. This protocol enables investigators to rapidly process archived FFPE tissue to study the epigenetic landscape in tumor samples and non-malignant controls.

To start, Covaris recommends users to perform a one-time titration of Proteinase K and a shearing time course study to empirically determine the optimal treatment conditions for your sample.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010464	A	12/18	As released
010464	B	07/19	Add proper reagent labels, consumable PNs, required holders/inserts; remove tissue/sectionPICK

PROCEDURE OVERVIEW



SAMPLE INPUT REQUIREMENTS

The truChIP FFPE Chromatin Shearing Kit performs optimally with at least two scrapings from 10 µm slides (~1cm² tissue/slide). The deparaffinization step uses the AFA technology to emulsify the paraffin in the microTUBE-130 Pre-slit Screw Cap followed by chromatin shearing in microTUBE-130 AFA Fiber Snap Cap.

This kit efficiently isolates high-quality soluble chromatin that can be immunoprecipitated (IP) for downstream application, such as ChIP-seq. The kit contains enough reagents and consumables to process up to 25 samples.

KIT CONTENTS

Tissue SDS Buffer	3 mL	1X Lysis Buffer
Proteinase K Solution	0.125 mL	10ng/ul Digestion Enzyme
Chromatin Shearing Buffer	2 mL	1X Shearing Buffer
Protease Inhibitor Cocktail	0.2 mL	100X Inhibitor Mix
microTUBE-130 ul Pre-slit Screw Cap	(25) microTUBE-130 AFA Fiber, Pre-slit Screw Cap (PN: 190322 in kit, PN: 520216 in Online Store)	For use with Paraffin Emulsification protocol
microTUBE-130 ul Pre-slit Snap Cap	(25) microTUBE-130 AFA Fiber, Pre-slit Snap Cap (PN: 520045)	For use with Chromatin Shearing protocol

Safety Data Sheets: http://covaris.com/resources/safety-data-sheets

STORAGE

The kit is shipped cold and should be stored at 2-8°C.

Note: Mix solutions well before use to ensure solutions are completely solubilized.

SUPPLIED BY USER

- Microcentrifuge (5,000 x g capability)
- Dry heating block such as Eppendorf ThermoMixer or similar with either 1.5 or 2 mL heat block inserts. We recommend two heating blocks, preset at 56°C and 80°C respectively and microTUBE-130 Centrifuge and Heat Block Adapter (PN: 500406)

Consumables

- 1.5 ml nuclease-free microfuge tubes (e.g., Eppendorf Safe-Lock Tubes, PN: 022363212)

Optional Supplies for Sample Collection from Slides

- Scalpel Blade #11 by Feather (e.g. World Precision Instruments, PN: 504170)
- Scalpel Handel (e.g. World Precision Instruments, PN: 500236)

FOCUSED-ULTRASONICATOR SETUP

M220 Holders/Inserts Required		
Consumable Part Number	Description	M220 Holder & Insert
520216	microTUBE-130 AFA Fiber, Pre-Slit Screw-Cap	500414 & 500420
520045	microTUBE-130 AFA Fiber Pre-Slit Snap-Cap	500414 & 500489
Position Holder XTU and Insert into place and fill the water bath until the water level reaches the top of the holder. After setting up the system, wait until the water bath has reached the set temperature.		

PROTOCOL

A. Paraffin Emulsification

1. Set up the dry-heat block and set to 40˚C . Insert the required number of microTUBE-130 centrifuge/heat block adapters into the heat block.
2. Add 100 µl of Tissue SDS Buffer to a microTUBE-130 Pre-Slit Screw-Cap
3. Capture regions of interest (ROIs) from two unstained 10µm slides (~1cm² tissue/slide) with a scalpel. We recommend a Feather Surgical Blade #11 or equivalent. This blade is thin enough it can be inserted into the microTUBE-130 Pre-Slit Screw-Cap making handling much easier. We recommend adding ~2 ul of Tissue Lysis buffer directly onto the slide/sample to allow that the FFPE "flakes" to be scraped from the slide. This will form a sticky bulb that can easily be transferred to the microTUBE-130 Pre-Slit Screw-Cap.
4. Release the tissue into the microTUBE-130 Pre-Slit Screw-Cap (blue cap).
5. Add 0.8 µl of Proteinase K to the microTUBE-130 (final concentration 80ng/µl) and pipette to mix.
6. Load the microTUBE-130 Pre-Slit Screw-Cap into the microTUBE-130 Centrifuge/Heat Block adapter.
7. Incubate at 40˚C for 10 minutes with mixing after 5 minutes of incubation.

Note: Depending on tissue type and quantity, we recommend testing different quantities of tissue and Proteinase K concentrations. When starting with 2 tissue scapings, we recommend performing an initial Proteinase K titration using 40, 80, 120, 160, and 200 ng/µl.

Please contact applicationsupport@covaris.com for further support.

8. Process the sample on a Covaris M220 using the following settings:
   - Time 5 min
   - Duty Factor 20%
   - Peak incident 75W
   - 200 cycles per burst
   - 20°C

Note: Please contact Covaris at applicationsupport@covaris.com if using another instrument than the Covaris M220

9. Add 1 µl of Protease Inhibitor Cocktail. From here proceed at 4°C.

B. Chromatin Shearing

1. Using the table below, prepare a sufficient volume of Chromating Shearing Buffer. A 15% excess volume is recommended when preparing this buffer.

Number of Samples	1X Shearing Buffer	100X PIC
1	46 µl	0.5 µl
6	276 µl	3 µl
12	552 µl	6 µl
25	1150 µl	12.5 µl
X	X 46 µl	X µl

* Calculations include 15% excess

1. Transfer 90 µl of the paraffin emulsified sample from the microTUBE-130 Pre-Slit Screw-Cap into a microTUBE-130 Pre-Slit Snap-Cap.

2. Adjust the final volume in the microTUBE-130 Pre-Slit Snap-Cap to 130 µl with Chromatin Shearing Buffer supplemented with Protease Inhibitor Cocktail (100x).

3. Process the sample on a Covaris M220 using the following settings:

   - Time course: 10, 15, 20, and 30 min (once you have determined the optimal processing time for your tissue of choice directly process with this one time point only)
   - Duty Factor 15%
   - Peak incident 75W
   - 200 cycles per burst
   - 7 °C

Note: Please contact Covaris at applicationsupport@covaris.com if using an instrument other than the Covaris M220.

4. Place the microTUBE-130 ul Pre-Slit Snap-Cap in the microTUBE-130 centifuge/heat block adapter that has been loaded into a microcentrifuge (fixed angle rotor) and centrifuge at 5000 x g for 5 minutes at 4°C and retain the supernatant.
5. Place the sample on ice and determine chromatin concentration using Qubit fluorometric quantification.
6. Transfer an appropriate amount of the sample to a clean tube and proceed with IP.

Note: For histone and CTCF ChIPs from FFPE tissue, we achieved satisfactory results starting with ~500 ng input chromatin. For most IP workflows, the chromatin sheared in Chromatin Shearing Buffer must be diluted before proceeding to IP step. Therefore:

(a) 10 mM Tris-HCl pH 8.1, 300 mM NaCl, 2% Triton with which you dilute your chromatin 1:1 before proceeding to IP.

(b) In case your starting chromatin concentration is low, simply add NaCl to a final concentration of 150 mM and Triton to a final concentration of 1% before proceeding to IP.

To check the efficiency of your shearing, reserve 25 µl of the sheared chromatin, see **Appendix A** for detailed instructions.

Storing sheared chromatin is not recommended.

Freezing sheared chromatin is not recommended. Freeze/thaw cycles reduce IP efficiency and reproducibility.

SUPPLEMENTAL MATERIAL

Appendix A: Chromatin Shearing Efficiency Analysis Protocol

1. Take a 25 µl aliquot of the sheared sample and transfer to 0.6 mL microcentrifuge tube.
2. Add 1 µl of RNase A (10 mg/mL) and incubate at 37 °C for 30 min.
3. Add 1 µl of Proteinase K (10 mg/mL) and incubate at 56 °C for 2 hours in a PCR cycler with a heated lid.
4. Purify DNA using a commercial column-based kit (*e.g.,* Qiagen QIAquick PCR Purification Kit, Cat. No. 28104)
5. Elute from column or resuspend pellet with 50 µl of elution buffer (10 mM Tris-HCl, pH 8.5).
6. Load 1 µl of purified DNA onto the DNA 12000 chip and run on the Agilent 2100 BioAnalyzer.

Appendix B: Additional Notes

1. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the tissue type, mass, amount of paraffin and duration of formalin fixation.
2. The Covaris process uses high intensity focused ultrasonic (HIFU) energy and as such is influenced by objects in the acoustic path from the transducer surface to the fluid sample. For example, particles and bubbles in the water bath may scatter the acoustic energy from the sample. Replace the bath water daily and ensure that appropriate time has been allowed for degassing and water bath temperature to stabilize prior to use of the instrument.
3. Bubbles in the sample fluid in the tube may diminish the acoustic dose effectiveness. Be sure to fill the tubes slowly with the recommended volumes and avoid the use of additional detergents that may induce foaming.