PROTOCOL

Covaris

truChIP® Chromatin Shearing Kit

Adaptive Focused Acoustics (AFA)-based chromatin shearing for ChIP-based applications

PN 520154 and PN 520127

Contents

INTENDED USE

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

INTRODUCTION

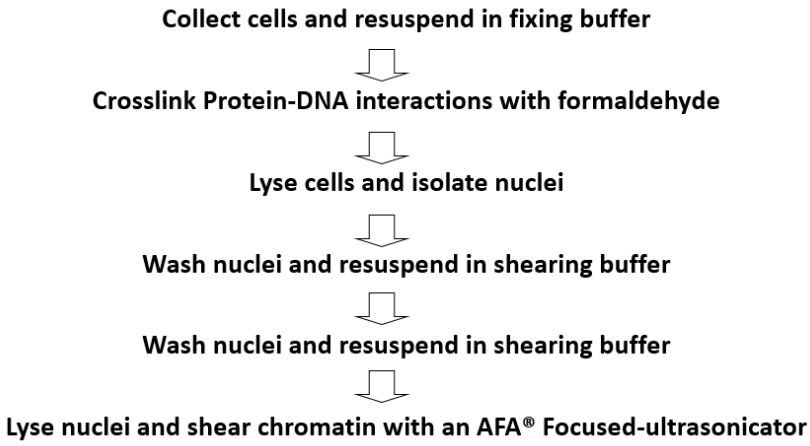
The truChIP Chromatin Shearing Kit is optimized for the efficient and reproducible shearing of chromatin from adherent and suspension mammalian cells using Covaris AFA™ Focused-ultrasonicators. Focused-ultrasonicators provide a non-contact and isothermal method of shearing chromatin without compromising the structural integrity of the target epitopes of interest. This kit can be used to prepare sample for ChIP-qPCR, ChIP-ChiP, and ChIP-Seq applications.

truChIP is compatible with a variety of cell types including primary, stem cells, and sorted cells. The Covaris shearing buffer contains SDS, which may not be compatible with immunoprecipitations (IP) using antibodies against some commonly used protein tags. Therefore, Covaris recommends contacting the antibody manufacturer prior to using this kit. To start, Covaris recommends users to perform a one-time fixation and shearing time course study to empirically determine the optimal treatment conditions.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010179	I	03/17	Update template and publish ME220 settings
010179	J	7/17	Remove specific content description of Buffer D3
010179	K	7/17	Correct procedure overview
010179	L	11/18	Correct step 10 on the nuclei prep protocol and add IP Dilution Buffer
010179	M	1/19	Add wash buffer preparation instructions

PROCEDURE OVERVIEW



SAMPLE INPUT REQUIREMENTS

The truChIP Chromatin Shearing Kit it is compatible with a range of inputs from less than 1 Million ($1x10^6$) and up to 200 Million ($2x10^8$) cells. The Low Cell protocol has been optimized for chromatin shearing of 1 to 3 Million Cells using the microTUBE-130 with AFA Fiber; the High Cell protocol uses the milliTUBE–1 mL with AFA Fiber to process up to 30 Million cells; and the Batch protocol is optimized for preparing up to 200 Million cells cultured as a suspension for chromatin shearing in 6 separate milliTUBE–1 mL with AFA Fiber.

Single Sample	Low Cell	High Cell	Batch
Input cell number	1 to 3 Million ($1\text{-}3 \times 10^6$) Cells	5 to 30 Million ($0.5\text{-}3 \times 10^7$) Cells	50 to 200 Million ($0.5\text{-}2 \times 10^8$) Cells
Number of samples sheared per kit	50	15	2
AFA tube	microTUBE–130	milliTUBE–1 mL with AFA Fiber	6 × milliTUBE–1 mL with AFA Fiber
Shearing volume	130 µl	1 mL	6 x 1 mL

KIT CONTENTS

Buffer A	7.5 mL	10X Fixing Buffer
Buffer B	5 mL	5X Lysis Buffer
Buffer C	2.5 mL	10X Wash Buffer
Buffer D3	6 mL	10X SDS Shearing Buffer
Buffer E	6 mL	1X Quenching Buffer
Buffer F	0.8 mL	100X Protease Inhibitor Cocktail
Dilution Buffer	20 mL	2X IP Dilution Buffer
Formaldehyde	5x1 mL ampules (PN 520154 only)	16% methanol-free formaldehyde

Note: Certain mammalian cell lines may have more proteases (nucleases), therefore, end-users can substitute **Buffer F** with other commercially available protease inhibitor cocktails.
Note: Because certain cell lines may have more nuclease activity, Covaris recommends adding EDTA to a final concentration of 1 mM in the 1X Covaris Wash Buffer C.

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

STORAGE

The kit is shipped cold and should be stored at 2 to 8C. Prior to use, kit reagent Buffers D3 and E may have to be warmed to 55C to dissolve precipitate and then cooled to room temperature before use.

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

SUPPLIED BY USER

- Molecular Biology Grade Water – Thermo Scientific (Cat. No. SH3053802), Mo Bio (Cat. No. 17012-200), or equivalent
- Phosphate Buffered Salt Solution (PBS) – Mo Bio (Cat. No. 17330-500), Thermo Scientific (Cat. No. SH30256.FS), or equivalent
- RNase A (DNase free) Thermo Scientific (Cat. No. EN0531) or equivalent
- Proteinase K (RNase and DNase free) Thermo Scientific (Cat. No. 17916), NEB (Cat. No. P8102S), or equivalent
- Covaris Focused Ultrasonicator (M220, ME220, S220, E220 Evolution, E220, or LE220)
- Refrigerated centrifuge having 15,000 x g capability
- Rocker – Nutator® or equivalent
- AFA tubes & holders/racks

Low Cell Protocol Consumables & Holders/Racks Required

Part Number	Description	M220 Holder & Insert	ME220 Holder & Insert	S-Series Holder	E220Evo Rack	E220 Rack	LE220 Rack
520045	microTUBE AFA Fiber Pre-Slit Snap-Cap	500414 & 500489	500514 & 500526	500114	500433	500111	NA
520052	microTUBE AFA Fiber Crimp-Cap	NA	500514 & 500526	500114	500433	500282	500282
520216	microTUBE-130 AFA Fiber Screw-Cap	500414 & 500489	500522 & 500534	500339	NA	NA	NA
520053	8 microTUBE Strip V1	NA	500514 & 500526	NA	500430	500191	500191
520217	8 microTUBE-130 AFA Fiber Strip V2	NA	500518 & 500526	NA	NA	NA	NA
520078	96 microTUBE Plate	NA	NA	NA	NA	No rack required	500329

High Cell Protocol Consumable & Holders/Racks Required

Part Number	Description	M220 Holder & Insert	ME220 Holder & Insert	S-Series Holder	E220Evo Rack	E220 Rack	LE220 Rack
520130	milliTUBE–1 mL with AFA Fiber	500414 & 500422	500520 & 500534	500371	500431	500368	500368

PROTOCOL

A. Cell Preparation and Crosslinking

The truChIP protocol uses a two-step lysis method to ensure reproducible and efficient shearing of both suspension and adherent cells. Follow the Cell Preparation and Crosslinking method (**A.1 – Suspension and A.2 – Adherent**) for your cell culture type.

Note: ChIP assays are sensitive to crosslinking and shearing conditions. Therefore, Covaris recommends users to include multiple fixation and shearing time points to empirically determine the optimal treatment conditions.

A.1 Suspension cells

1. Prepare solutions for the appropriate number of samples being processed before starting.

Buffer	Low Cell	High Cell	Batch
Cold 1X PBS	**Final Volume: 2.0 mL per sample** - Store on ice	**Final Volume: 4 mL per sample** - Store on ice	**Final Volume: 40 mL per batch** - Store on ice
1X Fixing Buffer A	**Final Volume: 0.5 mL per sample** - Mix 50 µl of Fixing Buffer A with 0.450 mL of molecular biology grade water	**Final Volume: 2 mL per sample** - Mix 200 µl of Fixing Buffer A with 1.8 mL of molecular biology grade water	**Final Volume: 20 mL per batch** - Mix 2.0 mL of Fixing Buffer A with 18 mL of molecular biology grade water
Fresh 11.1% Formaldehyde	**Final Volume: 1 mL per 1 to 20 samples** - Mix 690 µl of 16% Fresh Formaldehyde with 310 µl of molecular biology grade water	**Final Volume: 1 mL per 1 to 5 samples** - Mix 690 µl of 16% Fresh Formaldehyde with 310 µl of molecular biology grade water	**Final Volume: 2 mL per batch** - Mix 1.38 mL of 16% Fresh Formaldehyde with 0.62 mL of molecular biology grade water
Quenching Buffer E	Place in a 55C water bath to dissolve crystals, then place at ambient		
Important Notes • The use of fresh methanol-free formaldehyde is required to achieve reproducible results • The methanol-free formaldehyde ampule is for one-time use only–storage for later use is not recommended			

2. Collect cells by centrifugation at 200 x g for 5 minutes at room temperature. Remove media and wash cells once with cold 1X PBS and collect cells again by centrifugation.

> **Note:** Some cells do not pellet well at 200 x g. If a "spongy" pellet is not visible, increase speed at 100 x g intervals until a pellet is visible.

Reagent	Low Cell	High Cell	Batch
Cold 1X PBS	400 µl	1.5 mL	20 mL
Input cell number	1-3 x 10^6 Cells	1-3 x 10^7 Cells	0.5-2 x 10^8 Cells
Centrifuge Tube	2.0 mL	2.0 mL	50 mL

3. Re-suspend cells in room temperature Fixing Buffer A.

Reagent	Low Cell	High Cell	Batch
Fixing Buffer A	400 µl	1.5 mL	20 mL

4. Crosslink cells by adding freshly prepared 11.1% formaldehyde solution to a final concentration of 1% and set timer.

Reagent	Low Cell	High Cell	Batch
Fresh 11.1% Formaldehyde	40 µl	150 µl	2.0 mL

> **Note:** The use of fresh methanol-free formaldehyde is required to achieve reproducible results.

5. Place cells on a shaking platform at room temperature for the recommended time.

> **Note:** We recommend including two fixation times. Typically, **2.5 and 5 minutes** for stem and primary cells, and **5 and 10 minutes** for all other cell types.

6. Quench the crosslinking reaction by adding the appropriate volume of Quenching Buffer E to the fixed cells. Keep cells on rocker at room temperature for an additional 5 minutes.

Reagent	Low Cell	High Cell	Batch
Quenching Buffer E	23 µl	87 µl	1.2 mL

7. Collect cells by centrifuging at 500 x g for 5 minutes at room temperature.

8. Aspirate the supernatant and wash twice with cold 1X PBS.

Reagent	Low Cell	High Cell	Batch
Cold 1X PBS	300 µl	1.0 mL	5.0 mL

9. Collect cells by centrifugation at 200 x g for 5 minutes, 4C.

10. Proceed to nuclei preparation and chromatin shearing steps.

> **Note:** You may flash-freeze the fixed cells in liquid nitrogen at this point and store at -80C for short periods of time (*e.g.,* 2 to 3 days). Longer-term storage is not recommended.

A.2 Adherent cells

1. Grow the proper amount of cells to conduct a single ChIP assay or the initial time course until they are 80 to 90% confluent.

	35 mm Plate	60 mm Plate	100 mm Plate	150 mm Plate
Cell Density	~0.8 x 10^6	~2.0 x 10^6	~5.5 x 10^6	~15 x 10^6
Protocol	Low Cell	Low Cell	High Cell	High Cell
Number of Plates	1 to 3	1 to 2	2 to 5	1 to 2

Note: Cell densities provided above are estimates provided as a general guideline. Accurate cell densities for your cell lines should be determined.

2. Prepare solutions for the appropriate number of samples being processed fresh before starting.

Buffer	35 mm and 60 mm Dish	100 mm and 150 mm Plate
Cold 1X PBS	**Final Volume: 14 mL per Plate** - Store on ice	**Final Volume: 25 mL per Plate** - Store on ice
1X Fixing Buffer A	**Final Volume: 2 mL per Plate** - Mix 200 µl of Fixing Buffer A with 1.8 mL of molecular biology grade water	**Final Volume: 5 mL per Plate** - Mix 500 µl of Fixing Buffer A with 4.5 mL of molecular biology grade water
Fresh 11.1% Formaldehyde	**Final Volume: 300 µl per Plate** - Mix 208 µl of 16% Fresh Formaldehyde with 92 µl of molecular biology grade water	**Final Volume: 1 mL per Plate** - Mix 0.69 mL of 16% Fresh Formaldehyde with 0.31 mL of molecular biology grade water
Quenching Buffer E	Place in a 55C water bath to dissolve crystals, then place at ambient	
Important Notes • The use of fresh methanol-free formaldehyde is required to achieve reproducible results • The methanol-free formaldehyde ampoule is for one-time use only–storage for later use is not recommended		

3. Remove media and wash each plate one time with cold 1X PBS.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Cold 1X PBS	2 mL	5 mL

4. Remove PBS and add room temperature Fixing Buffer A to each dish.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Fixing Buffer A	2 mL	5 mL

5. Crosslink cells by adding freshly prepared 11.1% formaldehyde solution to a final concentration of 1% and start timing the crosslinking reaction.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Fresh 11.1% Formaldehyde	200 µl	500 µl

> **Note:** The use of fresh methanol-free formaldehyde is required to achieve reproducible results.

6. Place cells on a shaking platform at room temperature for the recommended time.

> **Note:** We recommend to test two fixation times. Typically, **2.5 and 5 minutes** for stem and primary cells, and **5 and 10 minutes** for all other cell types.

7. Quench the crosslinking reaction by adding the appropriate volume of Quenching Buffer E to fixed cells. Keep on a shaking platform at room temperature for an additional 5 minutes.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Quenching Buffer E	120 µl	300 µl

8. Completely aspirate the solution from the plate.

9. Add cold 1X PBS to each dish and scrape cells from the plate into a proper vessel.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Cold 1X PBS	450 µl	5 mL
Centrifuge Tube	2.0 mL tube	15 mL conical

10. Wash the plate with an additional volume of cold 1X PBS to collect any remaining cells.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Cold 1X PBS	450 µl	5 mL
Centrifuge Tube	2.0 mL tube	15 mL conical

11. Collect cells at 200 x g for 5 minutes, 4C.

> **Note:** Some cells do not pellet well at 200 x g. If a “spongy” pellet is not visible, increase speed at 100 x g intervals until a pellet is visible.

12. Wash cells twice by resuspending in cold 1X PBS, and collecting by centrifugation at 200 x g, 4C.

Reagent	35 mm and 60 mm Plate	100 mm and 150 mm Plate
Cold 1X PBS	450 µl	5 mL

13. Carefully and completely aspirate the supernatant from the tube(s), and place on ice. Proceed to nuclei preparation and chromatin shearing steps.

> **Note:** You may flash-freeze the fixed cells in liquid nitrogen at this point and store at -80C for short periods of time (*e.g.,* 2 to 3 days). Longer-term storage is not recommended.

B. Nuclei Preparation

1. Prepare the proper number of suspension or adherent cells according to **Step 1 on page 7**. Place the required number of AFA tubes on ice to pre-chill while preparing samples to shear.

	Low Cell	High Cell	Batch
Number of fixed cells	1-3 x 10^6	1-3 x 10^7	0.5- 2 x 10^8
AFA Tube	microTUBE-130	milliTUBE-1 mL	6×milliTUBE-1 mL

Note: Prepare the correct volume of fresh solutions for the nuclei preparation and chromatin shearing prior to beginning. Substituting any of the reagents or changing any steps will adversely affect shearing efficiency and reproducibility.

Buffer	Low Cell	High Cell	Batch
1X Lysis Buffer B	**Final Volume: 0.5mL per sample** - Mix 100 µl 5X Lysis Buffer B with 400 µl of molecular biology grade water - Add 5 µl of 100X Buffer F - Store on ice	**Final Volume: 1.0 mL per sample** - Mix 200 µl 5X Lysis Buffer B with 0.8 mL of molecular biology grade water - Add 10 µl of 100X Buffer F - Store on ice	**Final Volume: 10 mL per sample** - Mix 2 mL 5X Lysis Buffer B with 8 mL of molecular biology grade water - Add 100 µl of 100X Buffer F - Store on ice
1X Wash Buffer	**Final Volume: 0.5 mL per sample** - Mix 50 µl 10X Wash Buffer C with 450 µl of molecular biology grade water - Add 5 µl of 100X Buffer F - Optional: Add EDTA to a final concentration of 1mM - Store on ice	**Final Volume: 1.0 mL per sample** - Mix 100 µl 10X Wash Buffer C with 0.9 mL of molecular biology grade water - Add 10 µl of 100X Buffer F - Optional: Add EDTA to a final concentration of 1mM - Store on ice	**Final Volume: 10mL per sample** - Mix 1.0 mL 10X Wash Buffer C with 9 mL of molecular biology grade water - Add 100 µl of 100X Buffer F - Optional: Add EDTA to a final concentration of 1mM - Store on ice
1X Shearing Buffer D3 (1mM EDTA, 10mM Tris-HCl pH 7.6, 0.1% SDS)	**Final Volume: 1 mL per sample** - Mix 100 µl 10X Shearing Buffer D3 with 0.9 mL of molecular biology grade water - Add 10 µl of 100 X Buffer F - Store on ice	**Final Volume: 3 mL per sample** - Mix 300 µl 10X Shearing Buffer D3 with 2.7 mL of molecular biology grade water - Add 30 µl of 100 X Buffer F - Store on ice	**Final Volume: 10 mL per sample** - Mix 1.0 mL 10X Shearing Buffer D3 with 9.0 mL of molecular biology grade water - Add 100 µl of 100 X Buffer F - Store on ice

2. Add Lysis Buffer B containing 1× protease inhibitors to cross-linked cells to lyse plasma membrane, gently resuspend by aspirating/dispensing 4 times. If cells were frozen after formaldehyde fixation, thaw cells on ice first.

Reagent	Low Cell	High Cell	Batch
Lysis Buffer B	300 µl	1 mL	10 mL

3. If processing using Low Cell or High Cell volumes, then transfer to 1.5 mL microcentrifuge tube. If processing for Batch, then transfer to a 15 mL conical tube.

4. Incubate for 10 minutes on a rocker at 4C.

5. Collect intact nuclei by centrifugation at 1,700 x g for 5 minutes, 4C. Decant the supernatant without disturbing the nuclei pellet.

6. Gently resuspend pellet in Wash Buffer C containing protease inhibitor and incubate on a rocker for 10 minutes, 4C.

Reagent	Low Cell	High Cell	Batch
Wash Buffer C	300 µl	1 mL	10 mL

Note: The purpose of this wash is to significantly dilute the salts remaining from the Wash Buffer. Shearing in the presence of high salt concentrations may result in the reversal of formaldehyde cross-links during the process.

7. Collect nuclei by centrifugation at 1,700 x g for 5 minutes, 4C. Carefully remove and discard the wash solution, taking care not to disturb the nuclei pellet.

8. Gently rinse the sides of the tube with Shearing Buffer D3 containing Protease inhibitor. Slowly dispense the buffer down the entire circumference of the upper-inside of the tube, taking care not to disturb the nuclei pellet.

Reagent	Low Cell	High Cell	Batch
Shearing Buffer D3	300 µl	1 mL	1.5 mL

9. Collect nuclei by centrifugation at 1,700 x g for 5 minutes, 4C. Decant the supernatant without disturbing the nuclei pellet.

10. Repeat steps 8 and 9 an additional time. Carefully remove and discard the supernatant, taking care not to disturb the nuclei pellet.

C. Chromatin Shearing

1. Resuspend nuclei pellet in the Shearing Buffer D3 and transfer to appropriate AFA Tube(s).

Reagent	Low Cell	High Cell	Batch
Shearing Buffer D3	130 µl	1 mL	6 mL
AFA Tube	(1) microTUBE	(1) milliTUBE	(6) milliTUBE

2. Shear chromatin with an AFA Focused-ultrasonicator with appropriate rack or holder; settings are provided in **Appendix A**.

> **Note:** Optimization of shearing time should be conducted whenever experimental parameters (*e.g.,* cell type, cell number, or sample volumes) are changed.

3. If processing samples for Low Cell Chromatin Shearing Optimization in microTUBEs, please aliquot 130 µl of the nuclei preparation into 6 microTUBEs for carrying out the shearing time course. of 2, 4, 6, 8, 10, and 12 minutes.

   If processing samples for High Cell Chromatin Shearing Optimization, please aliquot 1 mL of the nuclei into one milliTUBE–1 mL with AFA Fiber for carrying out the shearing time course. of 2, 4, 8, 12, 15, and 20 minutes.

4. If processing samples using the milliTUBE–1 mL with AFA Fiber, you may process all time points of the time course study in the same tube according to the figure below. After each time point interval, remove 35 µl of the sample and place in in a pre-chilled microcentrifuge tube labeled with the total processing time – store on ice. Replace the removed volume with 35 µl of Covaris Shearing Buffer D3 before running the next time point.

Programmed Interval processing time (minutes)	2	2	4	4	3	5
Total processing time (minutes)	2	4	8	12	15	20

5. Place the milliTUBE in the holder/rack and process on the ultrasonicator for the next programmed interval processing time. 25 µl will be used for DNA shearing size range analysis, and 10 µl will be used for epitope integrity analysis using western. Replace the removed volume with 35 µl of Covaris Shearing Buffer D3 before running the next time point.

> **Note:** Carefully remove any traces of foam from the solution and the cap prior to placing sample back in the ultrasonicator for the next shearing time point when carrying out the time course experiment in the milliTUBE-1mL with AFA fiber.

6. After shearing, transfer samples into a pre-chilled microcentrifuge tube and place on ice until all tubes are processed. If batch processing using a high-throughput ultrasonicator (*e.g.*

E220), samples can be maintained in the instrument's water bath at 4C before and after processing.

Note: To check the efficiency of your shearing, reserve 25 µl of the sheared chromatin and see **Appendix B** for detailed instructions
Note: Sheared chromatin can be stored at 4C for up to 2 days
Note: Freezing sheared chromatin is not recommended. Freeze/thaw cycles reduce IP efficiency and reproducibility
Note: For subsequent immunoprecipitation, sheared chromatin can be diluted in the desired immunoprecipitation buffer. Alternatively, the composition of the shearing buffer can be adjusted appropriately for immunoprecipitation. **The 1× Shearing Buffer D3 composition is: 10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.1% SDS**

7. Before proceeding to IP, dilute the chromatin 1:1 with the Covaris 2X IP Dilution Buffer.

8. Centrifuge sample after the addition of the IP Dilution buffer is at 10,000 x g for 5 minutes at 4C to prepare the supernatant for IP.

Note: Sheared chromatin must be diluted prior to IP to sequester the SDS in Shearing Buffer D3 and ensure antibodies perform optimally. Therefore, for homebrewed IP methods, dilute the chromatin sheared with Covaris 1:1 with the 2X Dilution Buffer. Alternatively, if the chromatin sample should not be diluted further, add NaCl to a final concentration of 150mM and Triton to a final concentration of 1%.

SUPPLEMENTAL MATERIAL

Appendix A: AFA Focused-ultrasonicator Operating Conditions

Low Cell Chromatin Shearing Protocol						
Instrument	**M220**	**ME220**	**S220**	**E220 Evolution**	**E220**	**LE220**
Target Size (bp)	200-700	200-700	200-700	200-700	200-700	200-700
PIP	75	75	105	105	105	300
Duty Factor (%)	5	5	2	2	2	15
CPB	200	1000	200	200	200	200
Treatment Time (minutes)	2-20	2-20	2-12	2-12	2-12	2-12
Setpoint Temperature (C) [1]	7	9	6	6	6	6
Min/Max Temperature (C)	4/10	6/12	3/9	3/9	3/9	3/9
Max Cell Number (Million)	3M	3M	3M	3M	3M	3M
AFA Intensifier Required [2]	NA	NA	Integrated	Yes	Yes	NA
Water Level (run) [3]	Full	9	12	6	6	6
Sample Volume (µl) [4]	130	130	130	130	130	130

Important Notes

1. If using the S220, E220 Evolution, E220, or LE220, set the temperature on the external chiller 3C below the setpoint temperature for the run. The min/max is set in SonoLab
2. If intensifier is required, please ensure PN 500141 is used
3. Water level should always be 1mm below the neck of the microTUBE–130 cap
4. Always fill the microTUBE-130 with 130 µl of sample

High Cell Chromatin Shearing Protocol						
Instrument	**M220**	**ME220**	**S220**	**E220 Evolution**	**E220**	**LE220**
Target Size (bp)	200-700	200-700	200-700	200-700	200-700	200-700
PIP	75	75	140	140	140	420
Duty Factor (%)	10	15	5	5	5	30
CPB	200	1000	200	200	200	200
Treatment Time (minutes)	2-20	2-20	2-20	2-20	2-20	5-25
Setpoint Temperature (C) [1]	7	9	6	6	6	6
Min/Max Temperature (C)	4/10	6/12	3/9	3/9	3/9	3/9
Max Cell Number (Million)	30M	30M	30M	30M	30M	30M
AFA Intensifier Required	NA	NA	NA	NA	NA	NA
Water Level (run) [2]	Full	9	8	5	5	5
Sample Volume (mL) [3]	1	1	1	1	1	1

Important Notes

1. If using the S220, E220 Evolution, E220, or LE220, set the temperature on the external chiller 3C below the setpoint temperature for the run. The min/max is set in SonoLab
2. Water level should always be 1mm below the neck of the milliTUBE–1 mL with AFA Fiber cap
3. Always fill the milliTUBE–1 mL with AFA Fiber with 1.0 mL of sample

Appendix B: 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 37C for 30 min.
3. Add 1 µl of Proteinase K (10 mg/mL) and reverse crosslink by heating at 65C overnight in a PCR cycler with a heated lid.
4. Purify DNA using either a commercial column based kit (*e.g.,* Qiagen QIAquick PCR Purification Kit, Cat. No. 28104), or phenol-chloroform extraction and ethanol precipitation.
5. Elute from column, or resuspend pellet with 50 µl of elution buffer (10 mM Tris-HCl, pH 8.5).
6. Add 1 volume of loading dye to 5 volumes of purified DNA.

> **Note:** The use of loading dye without Bromophenol Blue is recommended. Bromophenol Blue migrates at ~300 bp and interferes with smear analysis.

7. Load 300 to 600 ng of purified DNA per lane.
8. Resolve on 1% agarose gel run at 30 V for 3.5 hours.
9. Stain gel with Ethidium Bromide after the gel is run.
10. Destain and view gel with a UV light source and record image.

> **Note:** Alternatively, 1 µl of purified DNA can be analyzed on an Agilent 2100 BioAnalyzer to provide a more accurate representation of the shearing size range and distribution.

Appendix D: Additional Notes

1. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the cell type and mass.
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 on a daily basis 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.

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