

Quick Guide

DNA Shearing with M220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using microTUBE-130, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE and a Covaris M220 Focused-ultrasonicator.

Values mentioned in this Quick Guide are nominal values. The tolerances are as follows:

- Temperature +/- 2 °C
- Sample volume
 - microTUBE-15: from 15 to 20 µl, +/- 1 µl
 - microTUBE-50: 55 µl, +/- 2.5 µl
 - microTUBE Snap-Cap: 130 µl, +/- 5 µl
 - microTUBE-500: 500 µl, +/- 10 µl or 320 µl, +/- 10 µl
 - miniTUBE: 200 µl, +/- 10 µl
- Water Level +/- 1

Sample Guidelines

- **DNA input:** up to 5 µg purified DNA (1 µg for the microTUBE-15; minimum 320 ng for the microTUBE-500)
- **Buffers:** Tris-EDTA, pH 8.0
- **DNA quality:** Genomic DNA (> 10 kb). For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.
- **WARNING:** DO NOT use the microTUBE or miniTUBE for storage. Samples should be transferred after processing.

Instrument Setup

- Refer to the instrument manual for complete setup.
- microTUBE and miniTUBE have specific holders associated with them.

Instrument Settings

- Recommended settings are subject to change without notice.
- Mean DNA fragment size distributions are based on electropherograms generated from the Agilent Bioanalyzer with DNA 12000 Kit (cat# 5067-1509), with the exception of the 320 µl microTUBE-500 protocol (High Sensitivity DNA Kit, cat# 5067-4626).

DNA fragment representation will vary with analytical systems, please carry out a time course based on settings provided in this document to reach desired fragment size distribution.

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130 µl Sample Volume: 150 to 1,500 bp

Vessel	microTUBE AFA Fiber Snap-Cap							
								
Part Number (PN)	520045							
Supported Sample Volume	130 µl							
Holder	M220 Holder XTU (PN 500414)							
Insert	M220 Holder XTU Insert microTUBE 130 µl (PN 500489)							
Temperature (°C)	20							
Target Base Pair Peak	150	200	300	400	500	800	1,000	1,500
Peak Incident Power (W)	50	50	50	50	50	50	50	50
Duty Factor (%)	20	20	20	10	10	5	2	2
Cycles per Burst (cpb)	200	200	200	200	200	200	200	200
Treatment Time (sec)	330	150	65	70	50	52	90	20

55 µl Sample Volume: 150 to 550 bp

Vessel	microTUBE-50 AFA Fiber Screw-Cap						
							
Part Number (PN)	520166						
Supported Sample Volume	55 µl						
Holder	M220 Holder XTU (PN 500414)						
Insert	M220 Holder XTU Insert microTUBE 50 µl (PN 500488)						
Temperature (°C)	20						
Target Base Pair Peak	150	200	250	300	350	400	550
Peak Incident Power (W)	75	75	75	75	75	75	25
Duty Factor (%)	10	10	10	10	10	10	10
Cycles per Burst (cpb)	200	200	200	200	200	200	500
Treatment Time (sec)	510	260	160	100	80	64	45

 **Even if the water level check button is green in SonoLab, please check that water is in contact with insert when using microTUBE-50. We recommend filling the water bath with 16 ml water.**

15 µl Sample Volume: 150 to 550 bp

Vessel	microTUBE-15 AFA Beads Screw-Cap			
				
Part Number (PN)	520145			
Supported Sample Volume	15 µl			
Holder	M220 Holder XTU (PN 500414)			
Insert	M220 Holder XTU Insert microTUBE 15 µl (PN 500420)			
Temperature (°C)	20			
Target Base Pair Peak	150	250	350	550
Peak Incident Power (W)	30	30	30	30
Duty Factor (%)	20	20	20	20
Cycles per Burst (cpb)	50	50	50	50
Treatment Time (sec)	250	80	42	23



To ensure reproducible DNA shearing, it is required to centrifuge samples before processing DNA in a microTUBE-15. Please see Appendix A for detailed instructions.

200 µl Sample Volume: 2,000, 3,000, and 5,000 bp

Vessel	miniTUBE		
			
Part Number (PN)	520064	520065	520066
Sample Volume	200 µl		
Holder	M220 Holder miniTUBE (PN 500302)		
Insert	N/A		
Temperature (°C)	7	20	20
Target Base Pair Peak	2,000	3,000	5,000
Peak Incident Power (W)	8	6	8
Duty Factor (%)	20	20	20
Cycles per Burst (cpb)	1,000	1,000	1,000
Treatment Time (sec)	900	600	600

320 µl and 500 µl Sample Volume: 150 to 600 bp

Vessel	microTUBE-500 AFA Fiber Screw-Cap				
					
Part Number (PN)	520185				
Holder	M220 Holder XTU (PN 500414)				
Insert	M220 Holder XTU Insert microTUBE-500 µl (PN 500471)				
Temperature (°C)	20				
Supported Sample Volume	320 µl	500 µl			
Target Base Pair Peak	500 to 600	150	200	350	550
Peak Incident Power (W)	75	75	75	75	50
Duty Factor (%)	20	20	20	20	20
Cycles per Burst (cpb)	200	200	200	200	200
Treatment Time (sec)	55	410	210	70	47

To fragment DNA to sizes larger than 5 kb, Covaris offers the g-TUBE: a single-use device that shears genomic DNA into selected fragments sizes ranging from 6 kb to 20 kb. The only equipment needed is a compatible bench-top centrifuge.

Additional Accessories

	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
	microTUBE-500 Screw-Cap Prep Station	500510
	miniTUBE Loading and Unloading Station	500207
Instrument Cleaning	M220 Fill & Drain Accessory Kit	500299
	M220 Swab Cleaning Kit	500298
AFA Grade Water	800 ml of AFA Grade Water	520101
Centrifuge and Heat Block microTUBE Screw-Cap Adapter	Fits microTUBE Screw-Caps into bench-top microcentrifuges	500406
g-TUBE	g-TUBEs (10) and prep station	520079

Appendix A: microTUBE-15 Centrifugation before DNA Shearing

1. Sample loading and centrifugation:

- microTUBE-15 AFA Beads Screw-Cap: Load and centrifuge microTUBE-15 Screw-Cap as described before placing the tubes in the rack.



Carefully load sample through septa making contact with the glass wall of the microTUBE.



Load microTUBE-15 into the centrifuge using microTUBE Adapter (PN 500406).



Balance centrifuge. Spin at 3000x g(RCF) for 30 seconds.

2. Sample Processing:

- Use settings provided on page 3.



Place the "Holder XTU Insert microTUBE-15 µl" (PN 500420) in the Holder XTU, you should feel the magnets contact.



Load microTUBE-15 into insert and lower the holder weight on top to keep the microTUBE in place.

3. Sample Recovery:



Place microTUBE-15 in Preparation Station and unscrew the cap.



Retrieve the sample with a narrow bore 20 µl pipet tip. It may be necessary to push the beads aside for full recovery.

Technical Assistance

- By telephone (+1 781.932.3959) during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at ApplicationSupport@covaris.com

Revision History

Part Number	Revision	Date	Description of Change
010252	G	1/2017	Update template; addition of microTUBE-500 AFA Fiber Screw-Cap protocol; update additional accessories; update Appendix C
010252	H	12/2018	Update microTUBE-50 protocols from 50 µl fill volume to 55 µl
010252	I	7/2019	Add protocol for using microTUBE-50 with Illumina TST-170 and TSO-170
010252	J	10/2019	Remove protocol for using microTUBE-50 with Illumina TST-170 and TSO-170; Reference new Shearing Guide, TruSight Tumor 170 DNA Shearing Quick Guide (PN 010515)