

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.


Table of Contents


microTUBE: 130 μ l Sample Volume: 150 to 1,500 bp	3
microTUBE-50: 55 μ l Sample Volume: 150 to 550 bp	4
microTUBE-50: 55 μ l Sample Volume: 185 bp for TST-170 and TSO-500	4
microTUBE-15: 15 μ l Sample Volume: 150 to 550 bp	5
miniTUBE: 200 μ l Sample Volume: 2,000, 3,000, and 5,000 bp	6
microTUBE-500: 320 μ l and 500 μ l Sample Volume: 150 to 600 bp	7
Additional Accessories	8
Appendix A	9
Technical Assistance	10
Revision History	10

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.

55 µl Sample Volume: 185 bp for Illumina TST-170 and TSO-500 Kits

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)	10							
Peak Incident Power (W)	25							
Duty Factor (%)	40							
Cycles per Burst (cpb)	1000							
Treatment Time (sec)	10							
Delay Time (s)	10							
AFA pulses (n)	10							

 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

		microTUBE-15 AFA Beads Screw-Cap			
Vessel					
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 μ l and 500 μ l 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 μ l (PN 500471)				
Temperature ($^{\circ}$ C)	20				
Supported Sample Volume	320 μ l	500 μ l			
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