

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.

Revision History

Part Number	Revision	Date	Description of change
010368	G	1/17	Update template; addition of microTUBE-500 AFA Fiber Screw-Cap protocol; update additional accessories; update Appendix C

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

- Temperature +/-2°C
- Sample volume
 - o microTUBE-15: from 15 to 20 µl, +/- 1 µl
 - o microTUBE-50: 50 µl, +/- 3.0 µl
 - o microTUBE Plate, Strip, Snap and Crimp Cap: 130 µl, +/- 5 µl or 50 µl, +/- 5 µl
 - o microTUBE-500: 500 µl, +/- 10 µl or 320 µl, +/- 10 µl
 - o 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)
- **Buffer:** 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.
- **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.

See http://www.covarisinc.com/wp-content/uploads/pn_010368.pdf for updates to this document.

130 µl sample volume - from 150 to 1,500 bp

	Vessel	microTUBE AFA Fiber Snap-Cap (PN 520045) 							
	Sample Volume	130 µl							
M220	Holder	M220 Holder XTU (PN 500414)							
	Insert	M220 Holder XTU Insert microTUBE 130 µl (PN 500489)							
	Temperature (°C)	20							
	Target BP (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	200	200	200	200	200	200	200	200
Treatment Time (s)	330	150	65	70	50	52	90	20	


50 µl sample volume - from 150 to 800 bp

	Vessel	microTUBE-50 AFA Fiber Screw-Cap (PN 520166) 							
	Sample Volume	50 µl							
M220	Holder	M220 Holder XTU (PN 500414)							
	Insert	M220 Holder XTU Insert microTUBE 50 µl (PN 500488)							
	Temperature (°C)	20							
	Target BP (Peak)	150	200	250	300	350	400	550	800
	Peak Incident Power (W)	75	75	75	75	75	75	75	75
	Duty Factor	10%	10%	10%	10%	10%	10%	10%	5%
	Cycles per Burst	200	200	200	200	200	200	200	200
Treatment Time (s)	360	195	130	90	70	60	40	50	



Even if the Water Level check button is green in SonoLab, please check that water is in contact with Insert when using microTUBE-50.


15 µl sample volume - from 150 to 550 bp

	Vessel	microTUBE-15 AFA Beads Screw-Cap (PN 520145)			
					
	Sample Volume	15 µl			
M220	Holder	M220 Holder XTU (PN 500414)			
	Insert	M220 Holder XTU Insert microTUBE 15 µl (PN 500420)			
	Temperature (°C)	20			
	Target BP (Peak)	150	250	350	550
	Peak Incident Power (W)	30	30	30	30
	Duty Factor	20%	20%	20%	20%
	Cycles per Burst	50	50	50	50
	Treatment Time (s)	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 - 2,000; 3,000 and 5,000 bp

	Vessel	miniTUBE		
		Clear (PN 520064)	Blue (PN 520065)	Red (PN 520066)
				
	Sample Volume	200 µl		
M220	Holder	M220 Holder miniTUBE (PN 500302)		
	Insert	N/A		
	Temperature (°C)	7	20	20
	Target BP (Peak)	2,000	3,000	5,000
	miniTUBE	Clear	Blue	Red
	Peak Incident Power (W)	8	6	8
	Duty Factor	20%	20%	20%
	Cycles per Burst	1000	1000	1000
	Treatment Time (s)	900	600	600

320 µl and 500 µl sample volume – from 150 to 600 bp

Vessel		microTUBE-500 AFA Fiber Screw-Cap (PN 520185)				
		320 µl		500 µl		
M220	Holder	M220 Holder XTU (PN 500414)				
	Insert	M220 Holder XTU Insert microTUBE-500 µl (PN 500471)				
	Temperature (°C)	20				
	Target BP (Peak)	500 - 600	150	200	350	550
	Peak Incident Power (W)	75	75	75	75	50
	Duty Factor	20%	20%	20%	20%	20%
	Cycles per Burst	200	200	200	200	200
	Treatment Time (s)	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

Technical Assistance

- By telephone (+1 781 932 3959) during the hours of 9:00am to 5:00pm, Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at techsupport@covarisinc.com

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 the 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

If some of the sample splashes onto the wall of the microTUBE while removing from centrifuge or placing into rack, repeat centrifuge step. All liquid should be at the bottom of the microTUBE-15 before starting the AFA treatment.

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