Quick Guide:

DNA Shearing with S220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using microTUBE-130, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE and a Covaris S220 Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010368	C	12/16	Correct PIP for miniTUBE Red (PN 520066)
010368	D	1/17	Addition of microTUBE-500 AFA Fiber Screw-Cap protocols; update 'Additional Accessories'; update Appendix A

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 Plate, Strip, Snap and Crimp Cap: 130 µl, +/- 5 µl or 50 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)
- **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 or racks 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) 				microTUBE AFA Fiber Crimp-Cap (PN 520052) 			
	Sample Volume	**130 µl**							
S220	**Holder**	S-Series Holder microTUBE (PN 500114)							
	Water Level	12							
	Temperature (°C)	7							
	Target BP (Peak)	**150**	**200**	**300**	**400**	**500**	**800**	**1,000**	**1,500**
	Peak Incident Power (W)	175	175	140	140	105	105	105	140
	Duty Factor	10%	10%	10%	10%	5%	5%	5%	2%
	Cycles per Burst	200	200	200	200	200	200	200	200
	Treatment Time (s)	430	180	80	55	80	50	40	15

55 µl sample volume - from 150 to 550 bp

	Vessel	microTUBE-50 AFA Fiber Screw-Cap (PN 520166) 						
	Sample Volume	**55 µl**						
S220	**Holder**	S-Series Holder microTUBE-50 Screw-Cap (PN 500492)						
	Water Level	10						
	Temperature (°C)	7						
	Target BP (Peak)	**150**	**200**	**250**	**300**	**350**	**400**	**550**
	Peak Incident Power (W)	100	75	75	75	75	75	50
	Duty Factor	30%	25%	20%	20%	15%	10%	10%
	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
	Treatment Time (s)	150	95	65	45	45	55	50

15 µl sample volume - from 150 to 550 bp

	Vessel	microTUBE-15 AFA Beads Screw-Cap (PN 520145) 				
	Sample Volume	**15 µl**				
S220	**Holder**	S-Series Holder microTUBE-15 Screw-Cap (PN 500427)				
	Water Level	15				
	Temperature (°C)	20				
	Target BP (Peak)	**150**	**200**	**250**	**350**	**550**
	Peak Incident Power (W)	18	18	18	18	18
	Duty Factor	20%	20%	20%	20%	20%
	Cycles per Burst	50	50	50	50	50
	Treatment Time (s)	300	120	80	45	22

To ensure reproducible DNA shearing, it is required to centrifuge samples before processing DNA in a microTUBE-15. Please see Appendix A for 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**		
S220	**Holder**	S-Series Holder miniTUBE (PN 500206)		
	Water Level	15		
	Temperature (°C)	7	20	20
	Target BP (Peak) miniTUBE	**2,000 Clear**	**3,000 Blue**	**5,000 Red**
	Peak Incident Power (W)	3	3	25
	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) 				
	Sample Volume	**320 µl**	**500 µl**			
S220	**Rack**	S220 Holder microTUBE-500 Screw-Cap (PN 500449)				
	Water Level	15				
	Temperature (°C)	7				
All	**Target BP (Peak)**	**500 - 600**	**150**	**200**	**350**	**550**
	Peak Incident Power (W)	75	175	175	175	175
	Duty Factor	25%	20%	20%	20%	5%
	Cycles per Burst	200	200	200	200	200
	Treatment Time (s)	75	400	180	55	110

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
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

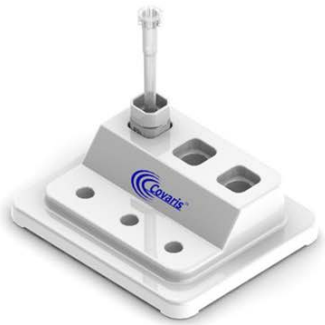
- By telephone (+1 781 932 3959) during the hours of 9:00am to 5:00pm, Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at techsupport@covarisinc.com

Appendix A – microTUBE-15 centrifugation before DNA Shearing

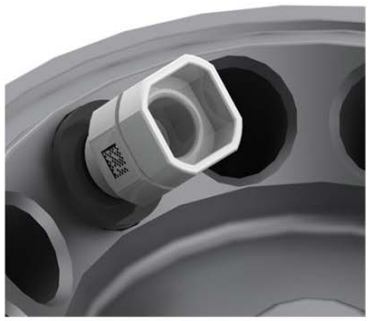
1. Sample loading and centrifugation

microTUBE-15 AFA Beads Screw-Cap

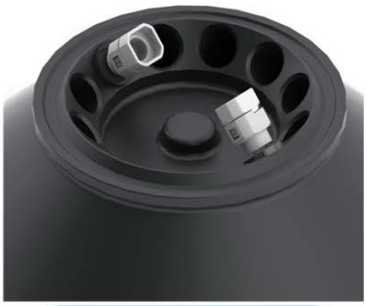
Load and centrifuge microTUBE-15 Screw-Cap as described before placing the tubes in the rack.



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.

3. Sample recovery

Repeat the centrifuge step before recovering sample from microTUBE-15.

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