

Quick Guide:

DNA Shearing with ME220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using oneTUBE-10, microTUBE-130, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE and a Covaris ME220 Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010349	E	4/18	Addition of 8 oneTUBE-10 AFA Strip protocols

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

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

Sample preparation guidelines

- **DNA input:** microTUBE-130 and microTUBE-50 up to 5 µg purified DNA; microTUBE-15 up to 1 µg; microTUBE-500 minimum 320 ng and up to 5 µg; oneTUBE-10 up to 100ng/µL concentration
- **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, miniTUBE, or oneTUBE for storage. Samples should be transferred after processing.**

Instrument setup


- Refer to the instrument manual for complete setup.
- DNA Shearing vessels have specific racks and waveguides associated with them.
- Water level settings are set by the rack definition and are not controlled by the user. Water level reading will be approximately 1-2mm higher when racks and tubes are submerged in acoustic bath.

Instrument settings





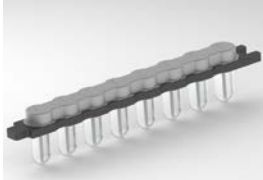
- Recommended settings are subject to change without notice.
- Mean DNA fragment size distributions are based on electropherograms generated from the Agilent Bioanalyzer with the DNA 12000 Kit (cat# 5067-1509), with the exception of the 320 µl microTUBE-500 protocol and the oneTUBE-10 (Agilent High Sensitivity DNA Kit, cat# 5067-4626). DNA fragment representation will vary with analytical systems, please carry out a time course experiment based on settings provided in this document to reach desired fragment size distribution.

See http://www.covarisinc.com/wp-content/uploads/pn_010349.pdf for updates to this document.



DNA Shearing with oneTUBE-10 - from 150 to 350 bp

Vessel	8 oneTUBE-10 AFA Strip PN 520225	
		
Rack	ME220 Rack 8 oneTUBE-10 AFA Strip PN 500609	
Wave Guide	PN 500526	
Water Level	8.5	
Water Temperature	20°C	
Sample Volume	10 µl	
Target BP (Peak)	175	350
Duration (s)	170	40
Peak Incident Power (W)	20	20
Duty Factor	25%	25%
Cycles per Burst	50	50
Sample Volume	50 µl	
Target BP (Peak)	175	350
Duration (s)	300	60
Peak Incident Power (W)	50	50
Duty Factor	25%	25%
Cycles per Burst	50	50



130 µl sample volume - from 150 to 550 bp

Vessel	microTUBE-130 AFA Fiber Screw-Cap (PN 520216) 	8 microTUBE-130 AFA Fiber Strip V2 (PN 520217) 8 microTUBE-130 AFA Fiber H Slit Strip V2 (PN 520239) 	
Rack	Rack 4-place microTUBE Screw-Cap PN 500522	Rack 8 microTUBE Strip V2 PN 500518	
Waveguide	PN 500534	PN 500526	
Sample Volume	130 µl	130 µl	
Water Level	9	9	
Water Temperature	20°C	20°C	
Target BP (Peak)	150 200 350 550	150 200 350 550	
Duration (s)	225 140 45 62	225 130 42 65	
Peak Power (W)	75 70 70 40	75 70 70 40	
Duty Factor (%)	25% 20% 20% 10%	25% 20% 20% 10%	
Cycles per Burst	1000 1000 1000 1000	1000 1000 1000 1000	
Vessel	microTUBE AFA Fiber Pre-Slit Snap-Cap (PN 520045) 	microTUBE AFA Fiber Crimp-Cap (PN 520052) 	8 microTUBE Strip V1 (PN 520053) 
Rack	Rack - Snap-Cap/Crimp-Cap/8 microTUBE Strip V1 PN 500514		
Waveguide	PN 500526		
Sample Volume	130 µl		
Water Level	6	7	6
Water Temperature	20°C	20°C	20°C
Target BP (Peak)	150 200 350 550	150 200 350 550	150 200 350 550
Duration (s)	225 130 42 65	240 140 45 65	225 130 38 55
Peak Power (W)	75 70 70 40	75 70 70 40	75 70 70 40
Duty Factor (%)	25 20 20 10	25 20 20 10	25 20 20 10
Cycles per Burst	1000 1000 1000 1000	1000 1000 1000 1000	1000 1000 1000 1000

55 μ l sample volume - from 150 to 550 bp

Vessel	<p>microTUBE-50 AFA Fiber Screw-Cap (PN 520166)</p> 	<p>8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)</p> <p>8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)</p> 
Rack	Rack 4-place microTUBE Screw-Cap PN 500522	Rack 8 microTUBE Strip V2 PN 500518
Waveguide	PN 500534	PN 500526
Sample Volume	55 μ l	55 μ l
Water Level	5.5	5.5
Water Temperature	20°C	20°C
Target BP (Peak)	150 200 350 550	150 200 350 550
Duration (s)	180 90 72 52	214 125 45 40
Peak Power (W)	75 75 50 25	50 50 50 50
Duty Factor (%)	25% 25% 10% 10%	30% 30% 20% 10%
Cycles per Burst	1000 1000 1000 1000	1000 1000 1000 1000




15 µl sample volume - from 150 to 550 bp

Vessel	microTUBE-15 AFA Beads Screw-Cap (PN 520145) 	8 microTUBE-15 AFA Beads Strip V2 (PN 520159) 8 microTUBE-15 AFA Beads H Slit Strip V2 (PN 520241) 
Rack	Rack 4-place microTUBE Screw-Cap PN 500522	Rack 8 microTUBE Strip V2 PN 500518
Waveguide	PN 500534	PN 500526
Sample Volume	15 µl	15 µl
Water Level	9.5	9.5
Water Temperature	20°C	20°C
Target BP (Peak)	150 200 350 550	150 200 350 550
Duration (s)	140 70 40 55	140 70 40 45
Peak Power (W)	50 50 30 18	50 50 30 15
Duty Factor (%)	30% 30% 20% 10%	30% 30% 20% 20%
Cycles per Burst	50 50 50 200	50 50 50 200




To ensure reproducible DNA shearing, it is required to centrifuge microTUBE-15 before processing. See Appendix B for instructions.

200 µl sample - 2,000; 3,000 and 5,000 bp

Vessel	miniTUBE Clear (PN 520064) 	miniTUBE Blue (PN 520065) 	miniTUBE Red (PN 520066) 
Rack	Rack 4 Place miniTUBE PN 500521		
Waveguide	PN 500534		
Sample Volume	200 µl		
Water Level	6		
Water Temperature	9°C	20°C	20°C
Target BP (Peak) miniTUBE	2,000 Clear	3,000 Blue	5,000 Red
Duration (s)	900	900	900
Peak Power (W)	8	8	10
Duty Factor (%)	20	20	25
Cycles per Burst	1000	1000	1000

320 µl sample - average fragment size 500 to 600 bp

Vessel	microTUBE-500 AFA Fiber Screw-Cap (PN 520185) 
Rack	Rack 4 Position microTUBE-500 PN 500525
Waveguide	PN 500534
Sample Volume	320 µl
Water Level	7
Water Temperature	20°C
Target BP (Peak)	500 - 600
Duration (s)	65
Peak Power (W)	75
Duty Factor (%)	20
Cycles per Burst	1000

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
	ME220 Rack Loading Station	500523
Centrifuge and Heat Block microTUBE Screw-Cap Adapter	Fits microTUBE Screw-Caps into bench top microcentrifuges	500406
Centrifuge 8 microTUBE Strip V2 Adapter	Fits the 8 microTUBE Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge	500541
g-TUBE	g-TUBEs (10) and prep station	520079
oneTUBE-10 Strip Caps	8 oneTUBE-10 AFA Strip Caps	500613

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 ApplicationSupport@covaris.com

Appendix A – 8 oneTUBE-10 AFA® Strip Sample loading and centrifugation

1. Fill the tubes

Aspirate sample and dispense into the 8 oneTUBE-10 AFA® Strip. Dispense the samples about 2-3 mm above the bottom of the tube or at the bottom depending on sample volume, being careful to dispense all the sample into the bottom of the tube.

2. Seal the plate for AFA-processing

Apply strip caps to tops of tubes. The strip is now ready to be processed in your Covaris instrument.

3. Centrifugation

We recommend centrifuging the strip after sample addition to ensure the sample is at the bottom of the tube. Centrifuge the strip using a mini-centrifuge compatible with 8-Strips for ≤ 10 seconds. Visually inspect strip to verify that all liquid is at the bottom of each tube before proceeding to Step 4.

4. Sample processing

The strip must be in the ME220 Rack 8 oneTUBE-10 AFA Strip (PN 500609) using the waveguide (PN 500526) for processing. Use settings provided on page 2.

5. Centrifugation

It is recommended to centrifuge the plate after AFA® (using a mini-centrifuge compatible with 8-Strips for ≤ 10 seconds) before removing any sample.

6. Downstream sample handling

After AFA® treatment, the samples are ready for downstream processing. The strip caps can be removed for processing in the 8 oneTUBE-10 AFA® Strip. Do not use the strip for long term storage of the samples.

Appendix B – 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.

8 microTUBE-15 AFA Beads Strip V2

The 8 microTUBE-15 AFA Beads Strip V2 will fit into the Covaris Centrifuge 8 microTUBE Strip V2 Adapter (PN 500541) for the Thermo Scientific™ mySPIN™ 12 mini centrifuge. Place the strip in the adapter and spin for a minimum of 1 minute.

2. Sample processing

Use settings provided on page 5.

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 μ L pipet tip. It may be necessary to push the beads aside for full recovery