

Quick Guide:

DNA Shearing with ME220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols for the Covaris oneTUBE-10, microTUBE, and miniTUBE consumables using a Covaris ME220 Focused-ultrasonicator instrument.

Revision History

Part Number	Revision	Date	Description of change
010349	G	1/2019	8 microTUBE-50 AFA Fiber Strip 150bp shearing protocol with SonoLab version 8.0.2 or higher
010349	H	2/2019	Update Quick Guide format. 8 microTUBE-50 AFA Fiber Strip 350bp shearing protocol with SonoLab version 8.0.2 or higher
010349	I	3/2019	Updated shearing protocol with SonoLab version 8.0.2 or higher

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

- Temperature +/-5°C
- Sample volume
 - o oneTUBE-10: 10 to 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 +/- 0.5

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 100 ng/µL concentration
- **Buffers:** TE - Tris-EDTA, pH 8.0 or truSHEAR™ Covaris (p/n 520248 or 520247). The Covaris TruSHEAR Buffer is optimized for AFA mechanical DNA Shearing. Formulated at 11x for DNA conditioning before processing (truSHEAR User Manual p/n 010400)
- **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 oneTUBE-10, microTUBE, or miniTUBE for long term sample 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.

Instrument settings

- Recommended settings are subject to change without notice.


- **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.

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oneTUBE-10 with SonoLab 8.0.2 or higher

Vessel	8 oneTUBE-10 AFA Strip	
		
Sample Volume	45 µL TE + 5 µL 11x truSHEAR buffer*	
Part Number (PN)	520225	
Rack	ME220 Rack 8 oneTUBE-10 AFA Strip PN 500609	
Rack Definition	8 oneTUBE-10 Strip PN 520225.2	
Waveguide	PN 500534	
Temperature (°C)	12	
Analytical System	Agilent HS NGS Fragment Kit (1-6000bp) cat# DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	40	12
Duration (s)	10	10
Peak Power (W)	20	20
Duty Factor (%)	45	40
Cycles per Burst (#)	50	50
Total Treatment Time per sample (s)	400	120

* Covaris truSHEAR Buffer optimized for AFA DNA Shearing at approximately ~1.1x final concentration. For example:



- 50 µL = 45 µL TE + 5 µL 11x truSHEAR.
- 40 µL = 36 µL TE + 4 µL 11x truSHEAR.
- 30 µL = 27 µL TE + 3 µL 11x truSHEAR.

microTUBE-50 with SonoLab 8.0.2 or higher

Vessel	8 microTUBE-50 AFA Fiber Strip V2	8 microTUBE-50 AFA Fiber H Slit Strip V2
		
Sample Volume	55 μ L	
Part Number (PN)	520174	520240
Rack	Rack 8 microTUBE Strip V2 PN 500518	
Rack Definition	8 microTUBE-50 H Slit Strip V2 PN 520240.2	
Waveguide	PN 500526	
Temperature ($^{\circ}$ C)	12	
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626	
Base Pair Mode (bp)	150	350
Repeat/Iterations (#)	23	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	50	50
Duty Factor (%)	30	20
Cycles per Burst (#)	1000	1000
Total Treatment Time per sample (s)	230	70




See Appendix C for screenshots of the protocols entered into SonoLab 8.0.2 or higher.

microTUBE-130 with SonoLab 8.0.2 or higher

	8 microTUBE-130 AFA Fiber Strip V2	8 microTUBE-130 AFA Fiber H Slit Strip V2	microTUBE-130 AFA Fiber Screw-Cap	
Vessel				
Sample Volume	130 μ L			
Part Number (PN)	520217	520239	520216	
Rack	Rack 8 microTUBE Strip V2 PN 500518		Rack 4-place microTUBE Screw-Cap PN 500522	
Rack Definition	8 microTUBE-130 H Slit Strip V2 PN 520239.2		4 microTUBE-130 Screw-Cap PN 520216.2	
Waveguide	PN 500526		PN 500534	
Temperature ($^{\circ}$ C)	12			
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626			
Base Pair Mode (bp)	150	350	150	350
Repeat/Iterations (#)	30	6	25	6
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (W)	75	70	75	70
Duty Factor (%)	25	20	25	20
Cycles per Burst (#)	50	1000	50	50
Total Treatment Time per sample (s)	300	60	250	60

See Appendix C for screenshots of the protocols entered into SonoLab 8.0.2 or higher.

microTUBE-15

Vessel	8 microTUBE-15 AFA Beads Strip V2	8 microTUBE-15 AFA Beads H Slit		microTUBE-15 AFA Beads Screw-Cap				
								
Sample Volume	15 µL							
Part Number (PN)	520159		520241		520145			
Rack	Rack 8 microTUBE Strip V2 PN 500518				Rack 4-place microTUBE Screw-Cap PN 500522			
Rack Definition	8 microTUBE-15 Strip V2 PN 520159				4 microTUBE-15 Screw-Cap PN 520145			
Waveguide	PN 500534				PN 500526			
Temperature (°C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	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.

* For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>



**HS NGS Fragment Kit (1-6000bp) DNF-474 is a registered trademark of Agilent.

microTUBE-50 with SonoLab 8.0.1* or lower

Vessel	8 microTUBE-50 AFA Fiber Strip V2	8 microTUBE-50 AFA Fiber H Slit Strip V2	microTUBE-50 AFA Fiber Screw-Cap					
Sample Volume	55 μ L							
Part Number (PN)	520174	520240	520166					
Rack	Rack 8 microTUBE Strip V2 PN 500518			Rack 4-place microTUBE Screw-Cap PN 500522				
Rack Definition	8 microTUBE-50 H Slit Strip V2 PN 520159	8 microTUBE-15 H Slit Strip V2 PN 520241	4 microTUBE-15 Screw-Cap PN 520145					
Waveguide	PN 500534			PN 500526				
Temperature ($^{\circ}$ C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	150	200	350	550	150	200	350	550
Duration (s)	214	125	45	40	180	90	72	52
Peak Power (W)	50	50	50	50	75	75	50	25
Duty Factor (%)	30%	30%	20%	10%	25%	25%	10%	10%
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000	1000	1000



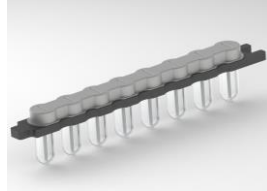
*For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>

microTUBE-130 with SonoLab 8.0.1* or lower

Vessel	8 microTUBE-130 AFA Fiber Strip V2	8 microTUBE-130 AFA Fiber H Slit Strip V2	microTUBE-130 AFA Fiber Screw-Cap					
								
Sample Volume	130 μ L							
Part Number (PN)	520217		520239		520216			
Rack	Rack 8 microTUBE Strip V2 PN 500518				Rack 4-place microTUBE Screw-Cap PN 500522			
Rack Definition	4 microTUBE-130 Screw-Cap PN 520216				4 microTUBE-130 Screw-Cap 520216			
Waveguide	PN 500526				PN 500534			
Temperature ($^{\circ}$ C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	150	200	350	550	150	200	350	550
Duration (s)	225	130	42	65	225	140	45	62
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


*For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>

microTUBE-130 with SonoLab 8.0.1* or lower

Vessel	microTUBE AFA Fiber Pre-Slit Snap-Cap	microTUBE AFA Fiber Crimp-Cap	8 microTUBE Strip V1									
												
Sample Volume	130 μ L											
Part Number (PN)	520045				520052				520053			
Rack	Rack - Snap-Cap/Crimp-Cap/8 microTUBE Strip V1											
Rack Definition	8 microTUBE-130 Snap-Cap PN 520045				8 microTUBE-130 Crimp-Cap PN 520052				8 microTUBE-130 Strip V1 PN 520053			
Waveguide	PN 500526											
Temperature ($^{\circ}$ C)	12											
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509											
Base Pair Mode (bp)	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

*For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>


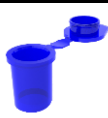

microTUBE-500

	microTUBE-500 AFA Fiber Screw-Cap
Vessel	
Sample Volume	320 µL
Part Number (PN)	520185
Rack	4 microTUBE-500 Screw-Cap PN 520185
Rack Definition	4 microTUBE-500 Screw-Cap PN 520185
Waveguide	PN 500534
Temperature (°C)	20
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626
Base Pair Mode (bp)	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.

* For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>

miniTUBE

	miniTUBE Clear	miniTUBE Blue	miniTUBE Red
Vessel			
Sample Volume	200 µL		
Part Number (PN)	520064	520065	520066
Rack	Rack 4 Place miniTUBE PN 500521		
Rack Definition	4 miniTUBE		
Waveguide	PN 500534		
Temperature (°C)	9	20	20
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509		
Base Pair Mode (bp)	2,000	3,000	5,000
miniTUBE	Clear	Blue	Red
Peak Power (W)	900	900	900
Duty Factor (%)	8	8	10
Cycles per Burst (#)	20	20	25
Duration (s)	1000	1000	1000

* For SonoLab version 8.0.2 or higher, refer to the next table. To upgrade SonoLab, visit our website: <https://covaris.com/resources/registered-users-login/m-series/>

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 strip 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



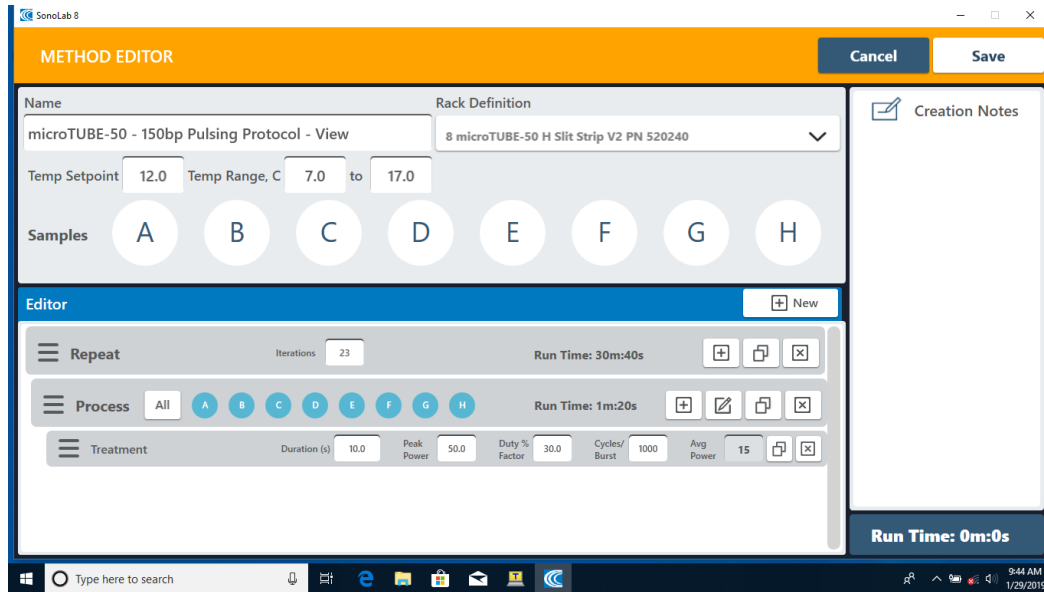
Retrieve the sample with a narrow bore 20 µL pipet tip. It may be necessary to push the beads aside for full recovery

Appendix C – Pulsing methods for microTUBE-50 in SonoLab version 8.0.2 or higher

Refer to the ME220 User Manual for detailed instructions for method creation.
https://covaris.com/wp-content/uploads/pn_010325.pdf

1. Method editor

As an example, below is a screenshot of the 150 bp protocol for the microTUBE-50 in the Method Editor with all samples selected. NOTE: Repeat (Iterations) is programmed before the Process.



2. Shearing protocol

Screenshot of the final shearing protocol with all samples selected for treatment.

