Quick Guide:

RNA Shearing with M220 and ME220 Focused-ultrasonicators

This Quick Guide provides recommendations for shearing single stranded Nucleic Acids (RNA, mRNA or ssDNA) with a Covaris M-Series Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010183	A	10/12	Creation of Fragmentation of Total RNA with M220 Protocol
010183	B	12/17	Updated template; addition of ME220 settings; addition of new consumables; updated additional accessories

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

- M220 Temperature +/-2°C. ME220 Temperature +/-5°C
- Sample volume
  - microTUBE-15: from 15 to 20 µl, +/- 1 µl
  - microTUBE-50: 55 µl, +/- 2.5 µl
  - microTUBE-130: 130 µl, +/- 5 µl
  - microTUBE-500: 500 µl, +/- 10 µl
- Water Level +/- 1

Sample guidelines

- **RNA input:** Up to 5 µg of total RNA or ssDNA. (1 µg for the microTUBE-15).
- **Buffer:** Tris-EDTA, pH 8.0.
- **RNA quality:** High quality total RNA, mRNA or ssDNA.

  DO NOT use the microTUBE for storage. Samples should be transferred after processing.

Instrument setup

- Refer to the instrument manual for complete setup.
- microTUBEs have specific holders or waveguides associated with them.

Instrument settings

- Recommended settings are subject to change without notice.
- Mean RNA fragment size distributions are based on electropherograms generated from the Agilent Bioanalyzer with RNA 6000 Nano Kit (cat# 5067-1511). RNA 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[(1)].

See http://www.covaris.com/wp-content/uploads/pn_010183.pdf for updates to this document.

(1) Treatment times are provided as a starting point for achieving 200 nt mean size. Due to the varaiblity in sample input, Covaris recommends setting up a time course experiment for determining appropriate treatment times (15% increments of time, up to +/- 50% of suggested treatment time). For total RNA treatment time should be increased, whilst for mRNA treatment time should be reduced. Please see Apendix A for example traces.

130 µl sample volume

	ME220		
Vessel	**microTUBE-130 AFA Fiber Screw-Cap (PN 520216)** 	**microTUBE AFA Fiber Pre-Slit Snap-Cap (PN 520045)** 	**8 microTUBE-130 AFA Fiber Strip V2 (PN 520217)** **8 microTUBE-130 AFA Fiber H Slit Strip V2 (PN 520239)** 
Sample Volume	**130 µl**	**130 µl**	**130 µl**
Rack	PN 500522	PN 500514	PN 500518
Waveguide	PN 500534	PN 500526	PN 500526
Water Level	9	6	9
Water Temperature	20°C	20°C	20°C
Peak Power (W)	70	70	70
Duty Factor	20%	20%	20%
Cycles per Burst	1000	1000	1000
Treatment Time (s)	~ 140(1)	~ 130(1)	~ 130(1)

	M220
Vessel	**microTUBE AFA Fiber Pre-Slit Snap-Cap (PN 520045)** 
Sample Volume	**130 µl**
Insert / Rack	PN 500489
Holder / Waveguide	PN 500414
Water Level	N/A
Water Temperature	20°C
Peak Power (W)	50
Duty Factor	20%
Cycles per Burst	200
Treatment Time (s)	~ 150(1)

Part Number: 010183 Rev B
Date: December 2017

50 µl sample volume

	M220	ME220	ME220
Vessel	**microTUBE-50 AFA Fiber Screw-Cap (PN 520166)** 		**8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)** **8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)** 
Sample Volume	**50 µl**	**55 µl**	**55 µl**
Insert / Rack	PN 500488	PN 500522	PN 500518
Holder / Waveguide	PN 500414	PN 500534	PN 500526
Water Level	N/A	5.5	5.5
Water Temperature	20°C	20°C	20°C
Peak Power (W)	75	75	50
Duty Factor	10%	25%	30%
Cycles per Burst	200	1000	1000
Treatment Time (s)	~ 195(1)	~ 90(1)	~ 125(1)

Even if the Water Level check button is green in SonoLab, please check that water is in contact with Insert when using microTUBE-50 in the M220.

Part Number: 010183 Rev B
Date: December 2017

15 µl sample volume

	M220	ME220	ME220
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)** 
Sample Volume	**15 µl**	**15 µl**	**15 µl**
Insert / Rack	PN 500420	PN 500522	PN 500518
Holder / Waveguide	PN 500414	PN 500534	PN 500526
Water Level	N/A	9.5	9.5
Water Temperature	20°C	20°C	20°C
Target (Peak)	**250**	**200**	**200**
Peak Power (W)	30	50	50
Duty Factor	20%	30%	30%
Cycles per Burst	50	50	50
Treatment Time (s)	~ 80(1)	~ 70(1)	~ 70(1)

To ensure reproducible DNA shearing, it is required to centrifuge samples before processing DNA in a microTUBE-15. Please see Appendix B for detailed instructions.

Part Number: 010183 Rev B
Date: December 2017

500 µl sample volume

	M220
Vessel	**microTUBE-500 AFA Fiber Screw-Cap (PN 520185)** 
Sample Volume	**500 µl**
Insert	PN 500471
Holder	PN 500414
Water Temperature	20°C
Peak Power (W)	75
Duty Factor	20%
Cycles per Burst	200
Treatment Time (s)	~ 210[(1)]

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
Instrument cleaning	M220 Fill & Drain Accessory Kit	500299
	M220 Swab Cleaning Kit	500298
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

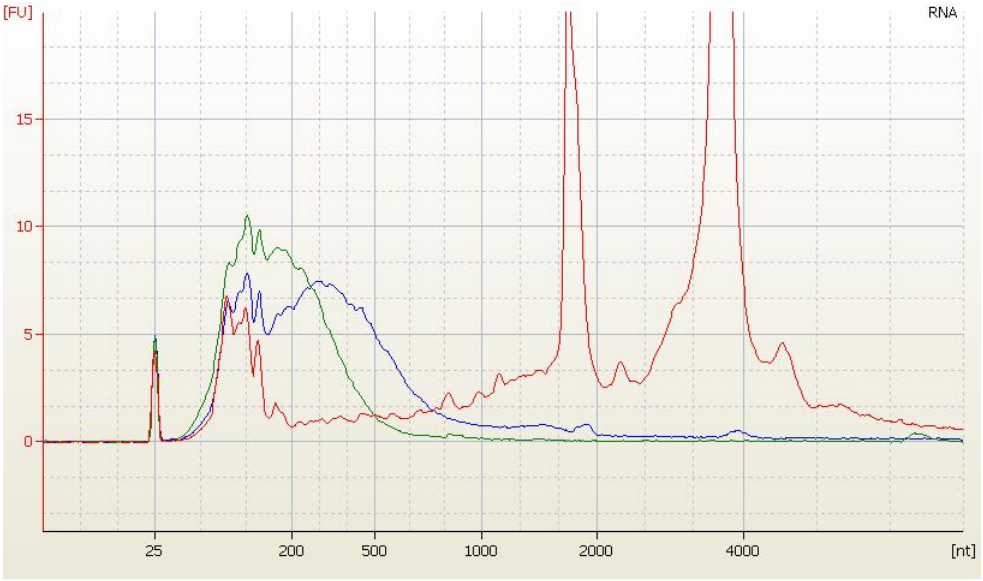
Technical Assistance

- 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

Part Number: 010183 Rev B
Date: December 2017

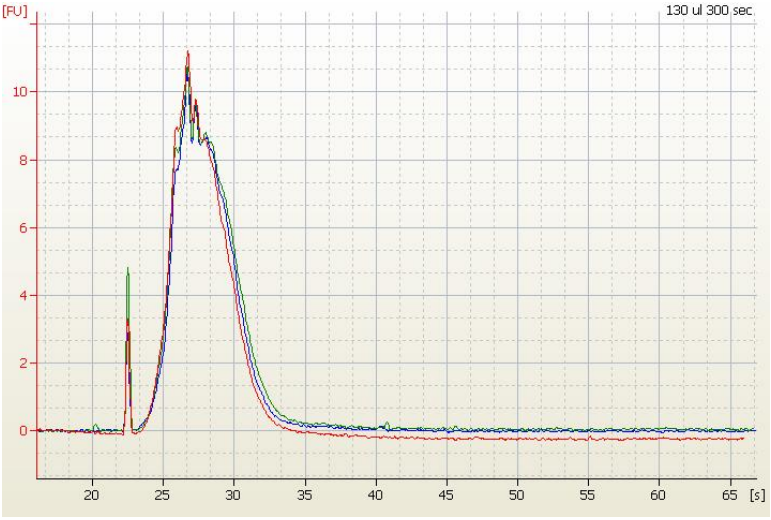
Appendix A – Representative fragmented RNA profiles

Figure 1 - Time course of total RNA fragmentation using Covaris M220 Focused-ultrasonicator



130 µl samples of total RNA were treated in the microTUBE AFA Fiber Snap-Cap using the following conditions: DF 20%, PIP 50W, CpB 200 for, 0 sec (Red trace), 60 sec (Blue trace), and 240 sec (Green trace). RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit.

Figure 2 - Reproducibility of RNA fragmentation using Covaris M220 Focused-ultrasonicator



In three separate experiments 130 µl RNA samples were sheared in a microTUBE AFA Fiber Snap-Cap using the following AFA conditions: DF 20%, PIP 50W, CpB 200 for 240 sec. Fragmented RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit.

Part Number: 010183 Rev B
Date: December 2017

Appendix B – microTUBE-15 centrifugation before RNA 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 at 1500rpm in the Thermo Scientific™ mySPIN™ 12 mini centrifuge.

2. Sample processing

Use settings provided on page 4.

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

Part Number: 010183 Rev B
Date: December 2017