

Quick Guide:

DNA Shearing with ME220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using 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 | B | 1/17 | Updated to include microTUBEs (Snap-Cap, Crimp-Cap, Strip V1), microTUBE-500 Screw-Cap, and miniTUBEs (Clear, Blue, Red) |
| 010349 | C | 3/17 | Addition of 8 microTUBE-130 AFA Fiber H Slit Strip V2, 8 microTUBE-50 AFA Fiber H Slit Strip V2, and 8 microTUBE-15 AFA Beads H Slit Strip V2 |

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

- Temperature +/-5°C
- Sample volume
 - 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:** 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





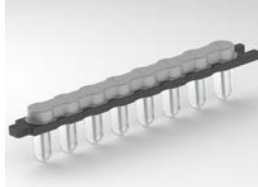
- Refer to the instrument manual for complete setup.
- DNA Shearing vessels have specific racks and waveguides associated with them.
- Water level settings are set by the rack definition and are not controlled by the user. Water level reading will be approximately 1-2mm higher when racks and tubes are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



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 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 µl 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 µl | 55 µl | | | | | | |
| Water Level | 5.5 | 5.5 | | | | | | |
| Water Temperature | 9°C | 9°C | | | | | | |
| Target BP (Peak) | 150 | 200 | 350 | 550 | 150 | 200 | 350 | 550 |
| Duration (s) | 170 | 78 | 75 | 45 | 214 | 125 | 45 | 40 |
| Peak Power (W) | 75 | 75 | 50 | 40 | 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 A 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) | 2,000 | 3,000 | 5,000 |
| miniTUBE | Clear | Blue | 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 |

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.

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 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