DNA Shearing Quick Guide:

microTUBE-15 with S220/E220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using microTUBE-15 and a Covaris S220 or E220 Focused-ultrasonicator.

Please note that microTUBE-15 requires removal of the Intensifier (PN 500141) from the E220 focused-ultrasonicator. Please see Appendix A on page 4 for instructions.

15 µL sample volume
microTUBE-15 AFA Beads Screw-Cap - from 150 to 550 bp
8 microTUBE-15 AFA Beads Strip V2 - from 150 to 550 bp

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
Temperature (°C)	20	20	20	20	20
Water Level – S220	15	15	15	15	15
Water Level – E220	10	10	10	10	10
Sample volume (µl)	15	15	15	15	15
E220 - Intensifier (PN 500141)	No	No	No	No	No

To ensure reproducible DNA shearing, it is required to centrifuge samples before processing.

1. Sample loading and centrifugation

Individual microTUBE-15

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 520059)

Balance centrifuge. Spin at 3000x *g* (RCF) for 1 minute

Carefully load microTUBE-15 into “S-Holder Screw-Cap LV”. If some of the sample splash up on the wall of the microTUBE while snapping the microTUBE-15 in the Holder, then repeat the centrifugation step. All the liquid should be at the bottom of the microTUBE-15 before to start the AFA treatment.

Rack of microTUBE-15 (E220)

Load and centrifuge microTUBE-15 as described above before placing the tubes in the Rack.

Rack of 8 microTUBE-15 Strip V2 (E220)

Place the 8 microTUBE-15 Strip V2 in the rack and load sample. Then centrifuge the rack at 300 RCF for 1 minute in a swinging bucket centrifuge.

2. Sample processing

Use settings provided in page 1

3. Sample recovery

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

Supplies

Covaris AFA Tubes	microTUBE-15 AFA Beads Screw-Cap (25) 8 microTUBE-15 AFA Beads Strip V2 (12)	520145 520159
Preparation station	microTUBE Prep Station Snap & Screw Cap	500330
Centrifuge Adapter	Centrifuge microTUBE Adapters (25)	520059
Holder for S220	Holder Screw-Cap LV	500427
Rack for E220	Rack 24 Place microTUBE Screw-Cap Rack 12 Place 8 microTUBE Strip V2	500308 500444
Starter Kits	S220 NGS microTUBE-15 Starter Kit Includes: Holder Screw-Cap LV microTUBE-15 AFA Beads Screw-Cap (25) Centrifuge microTUBE Adapters (25) microTUBE Prep Station Snap & Screw Cap	500418
	E220 NGS 24 microTUBE-15 Starter Kit Includes: Rack 24 Place microTUBE Screw-Cap microTUBE-15 AFA Beads Screw-Cap (25) Centrifuge microTUBE Adapters (25) microTUBE Prep Station Snap & Screw Cap	500419
	E220 NGS 96 microTUBE-15 Starter Kit Includes: Rack 12 Place 8 microTUBE Strip V2 8 microTUBE-15 AFA Beads Strip V2 (12)	500446

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

- Temperature +/-2°C
- Sample volume: from 15 to 20 µL, +/- 1µL
- Water Level +/- 1

Sample preparation guidelines

- **DNA input:** From 100 ng to 1 µg purified DNA
- **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.

Recommended settings are subject to change without notice.
See following link http://www.covarisinc.com/resources for updates to this document

Appendix A

TITLE: Removing or Installing the Intensifier (Covaris PN 500141) from an E System

The 500141 Intensifier is a small inverted stainless steel cone centered over the E Series transducer by four stainless wires. The wires are held by in a black plastic ring pressed into the transducer well.

If an AFA protocol requires "no intensifier", please *remove the Intensifier*, using the following steps:

1. Empty the water bath. Start the E System and start the SonoLAB software.
2. Wait for the homing sequence to complete (the transducer will be lowered with the rack holder at it home position, allowing easy access to the Intensifier).
3. Grasp opposite sides of plastic ring and gently pull the entire assembly out of the transducer well. Do not pull on the steel cone or the wires. The ring is a friction fit in the well – no hardware is used to hold it in place.

The 500141 Intensifier (left) shown installed in the E System transducer well and (right) removed.
Note the "UP" marking at the center of the Intensifier.

If a protocol requires the Intensifier to be present, simply reverse this process:

1. Align the black plastic ring with the perimeter of the transducer well. Note that the flat side of the center cone (marked UP) should be facing up (away from the transducer).
2. Gently press each section of the ring into the well until the ring is seated uniformly in contact with the transducer, with approximately 2 mm of the ring evenly exposed above the transducer assembly. Do not press on the cone or wires. The rotation of the ring relative to the transducer assembly is not important.
3. Refill the tank. Degas and chill the water before proceeding.

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