

## Quick Guide:

# DNA Shearing with LE220-plus Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using the 96 oneTUBE-10 AFA® Plate and a Covaris LE220-plus Focused-ultrasonicator.

### Revision History

Part Number	Revision	Date	Description of change
010433	A	12/17	Release of shearing guide with 96 oneTUBE-10 AFA® Plate protocols (PN 520249)
010433	B	2/18	Update of 10 µl shearing protocols for oneTUBE-10 (PN 520249): Timing for 150 and 350 base pair
010433	C	3/18	Update of 10 µl shearing protocols and 50µL shearing protocols for oneTUBE-10 (PN 520249): Timing for 150 and 350 base pair

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

- Temperature
  - o 20°C +/-2°C
- Sample volume
  - o 10 µl +/- 2 µl
  - o 50µL +/- 2 µL

### Sample preparation guidelines

- **DNA input:** up to 100ng/µl
- **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 oneTUBE-10 for long term sample storage.**

### Instrument setup

- Refer to the instrument manual for complete setup.

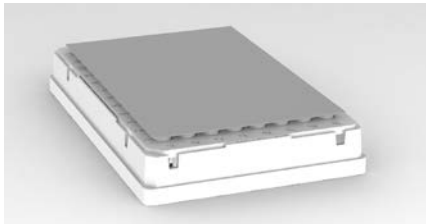

### Instrument settings

- Recommended settings are subject to change without notice.
  - Mean DNA fragment size distributions are based on peak analysis of fragments generated on a Fragment Analyzer (AATI) using the High Sensitivity NGS Fragment Kit (DNF-474). 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.covaris.com/wp-content/uploads/pn\\_010433.pdf](http://www.covaris.com/wp-content/uploads/pn_010433.pdf) for updates to this document.



**To ensure reproducible DNA shearing, it is recommended to centrifuge samples before processing DNA. Please see Appendix A for instructions.**

## 150 to 350 bp

<b>Consumable</b>	<b>96 oneTUBE-10 AFA® plate PN 520249</b> 	
<b>Instrument</b>	LE220-plus	
<b>Plate Definition</b>	"LE220plus_520249 96 oneTUBE Plate -2.2mm offset"	
<b>Water Level</b>		
<b>Rack</b>	Rack 96 oneTUBE-10 AFA® plate PN 500588	
<b>X and/or Y-dithering</b>	Yes 1mm Y-dither at 20mm/s	
<b>Temperature (°C)</b>	20	
<b>Sample Volume</b>	<b>10 µl</b>	
<b>Target BP (Peak)</b>	<b>175</b>	<b>350</b>
<b>Peak Incident Power (W)</b>	200	200
<b>Duty Factor</b>	25%	25%
<b>Cycles per Burst</b>	50	50
<b>Treatment Time/Duration (s)</b>	200	40
<b>Sample Volume</b>	<b>50 µl</b>	
<b>Target BP (Peak)</b>	<b>175</b>	<b>350</b>
<b>Peak Incident Power (W)</b>	200	200
<b>Duty Factor</b>	25%	25%
<b>Cycles per Burst</b>	50	50
<b>Treatment Time/Duration (s)</b>	390	85

### Additional Accessories

Product Description	Part Number
Thin foil seal	520235

## Appendix A –Sample loading and centrifugation

### Recommended Sequence for Use:

#### 1. Fill the tubes:

Aspirate sample and dispense into the 96 oneTUBE-10 AFA® Plate. 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:

Remove the backing from the thin foil seal and carefully align it over the plate. Using a sealing paddle or a roller (or your fingers), thoroughly press the seal on the tubes verifying that the seal is adhered to the top of each tube. The plate is now ready to be processed in your Covaris instrument.

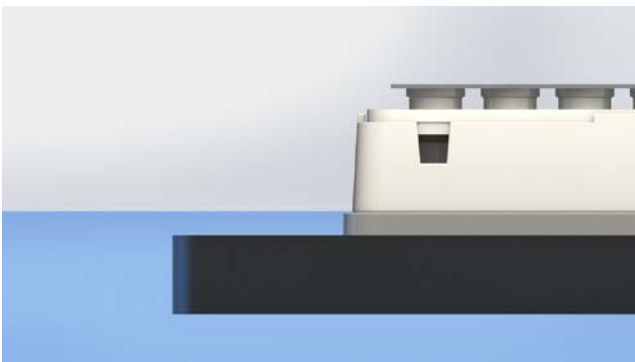
#### 3. Centrifugation:

We recommend centrifuging the plate after sample addition to ensure the sample is at the bottom of the tube. Centrifuge the plate at up to 7,500 RPM in a benchtop centrifuge compatible with 96 well plates for  $\leq 10$  seconds. **DO NOT STACK PLATES IN CENTRIFUGE.** Visually inspect plate to verify that all liquid is at the bottom of each tube before proceeding to Step 4.

#### 4. AFA-processing:

The plate must be in the Rack 96 oneTUBE-10 AFA® plate (PN 500588) for processing.

Check that the water is filled to the top of the edge of the bottom flange as depicted below:



#### 5. Centrifugation:

It is recommended to centrifuge the plate after AFA® (up to 7,500g (RCF) for  $\leq 10$  seconds) before removing any sample. **DO NOT STACK PLATES IN CENTRIFUGE.**

#### 6. Downstream sample handling:

After AFA® treatment, the samples are ready for downstream processing. The thin foil seal can be removed for processing in the 96 oneTUBE-10 AFA® Plate. Do not use the plate for long term storage of the samples.

## Technical Assistance

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- 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](mailto:ApplicationSupport@covaris.com)