

Quick Guide

DNA Shearing with R230 Focused-ultrasonicator

For Research Only

Software: SonoLab 10



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This Quick Guide provides DNA Shearing protocols for the Covaris R230 Focused-ultrasonicator instrument and associated consumables.

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

- *Temperature*. +/- 3 °C
- Sample Volume.
 - 96 AFA-TUBE TPX Plate for ≥ 300 bp: 50 μL, ± 2.5 μL
 - 96 AFA-TUBE TPX Plate for ≤ 300 bp: 20 μL, ± 2.5 μL
 - 384 AFA-TUBE 5 PP Plate 3.5 μL, ± 1.5 μL
- *Water Level*: +/- 0.5

Sample Guidelines

- DNA Input.
 - 96 AFA-TUBE TPX Plate up to 5 μg purified DNA
 - 384 AFA-TUBE 5 PP Plate up to 350 ng purified DNA
- Buffers. TE 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.
- **WARNING**. DO NOT use the 96 AFA-TUBE TPX Plates or 384 AFA-TUBE 5 PP Plates for long-term sample storage. Samples should be transferred after processing.

Instrument Setup

- Refer to the instrument manual for complete setup.
- Check for SonoLab™ updates and use the latest available version.
- DNA Shearing vessels have specific racks and waveguides associated with them.

Instrument Settings

• Recommended settings are subject to change without notice.

NOTE: DNA fragment representation will vary with analytical systems. Covaris recommends a time course experiment to reach the desired fragment size distribution. Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for time course experiment and for final optimized shearing.

Follow this link for updates to this document.



96 AFA-TUBE TPX Plate with SonoLab 10.0.0 or Higher

Vessel	96 AFA-TUBE TPX	Plate (PN 520291)
Suggested Sample Volume	5 to !	50 μL
Rack	R230 Rack AFA-TUBE TPX Plate (PN 500668)	
Plate Definition	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset	
Dithering	3 mm Y at 20 mm/s	
Temperature (°C)	10	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	13	5
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	280	280
Duty Factor (%)	25	25
Cycles per Burst (#)	50	50
Delay* Duration (sec)	10	10
Total Treatment Duration (sec)	130	50
Sample Volume (μL)	20	50

^{*}NOTE: The Delay step is required only for time course experiments and in run scenarios when only 1 column is being tested. For shearing in more than one column with optimized final setting, delay step is not required.

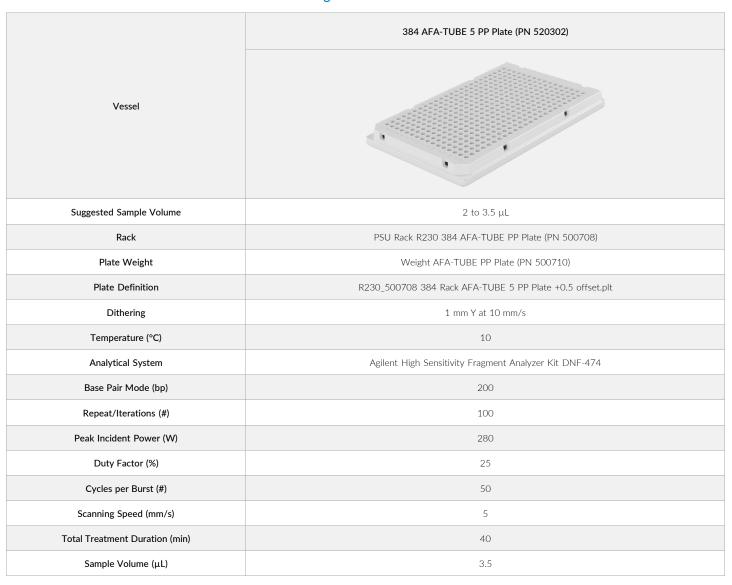
Please contact <u>ApplicationSupport@covaris.com</u> regarding details on how to set up a method for time course experiment and for final optimized shearing.

Additional Accessories

Product	Part Number	Product Description
R230 Rack AFA-TUBE TPX Plate	500668	This rack is compatible for use with the 96 AFA-TUBE TPX Plate on the R230 Focused-ultrasonicator



384 AFA-TUBE 5 PP Plate with SonoLab 10.0.1 or Higher



NOTE: Scanning will treat all samples of the plate in parallel. Please see Appendix E for examples of a Scanning Method in SonoLab 10.0.1 or higher.

Please contact <u>ApplicationSupport@covaris.com</u> regarding details on how to set up a method for time course experiment and for final optimized shearing.

Additional Accessories

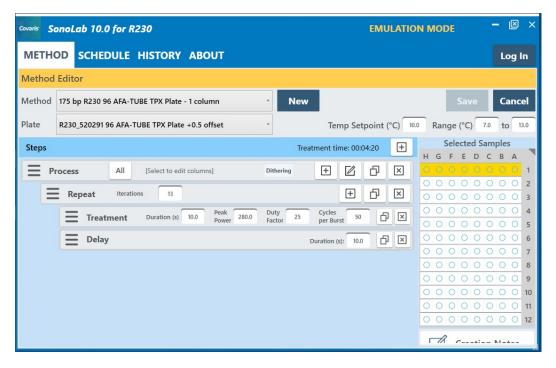
Product	Part Number	Product Description
PSU Rack R230 384 AFA-TUBE 5 PP Plate	500708	This rack is compatible for use with the 384 AFA-TUBE 5 PP Plate on R230 Focused-ultrasonicator
Weight AFA-TUBE PP Plate	500710	This weight is required for use with the 384 AFA-TUBE 5 PP Plate on R230 Focused-ultrasonicator

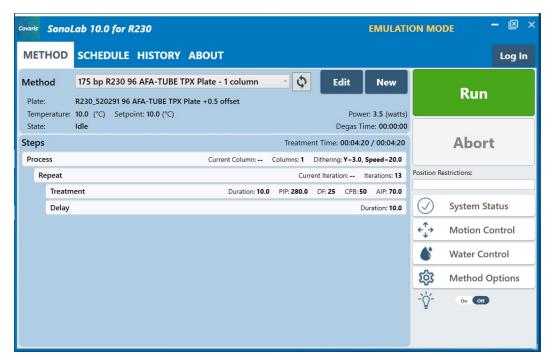


Appendix A: 175 bp Protocol on SonoLab 10.0.0 or Higher for R230 - 1 column

Below are some example screenshots for processing of 175 bp protocol with 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is added when processing only 1 column.

Shearing Protocol in Method Editor



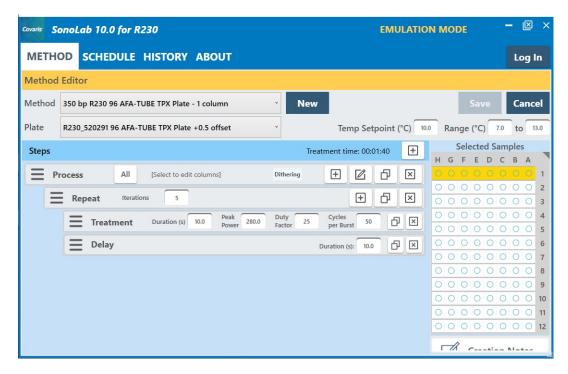




Appendix B: 350 bp Protocol on SonoLab 10.0.0 or Higher for R230 - 1 column

Below are some example screenshots for processing of 350 bp protocol with 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is added when processing only 1 column.

Shearing Protocol in Method Editor



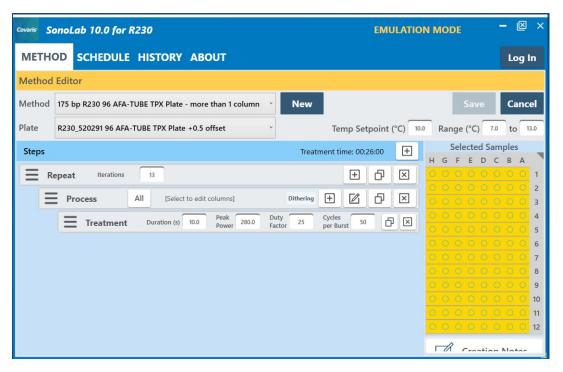


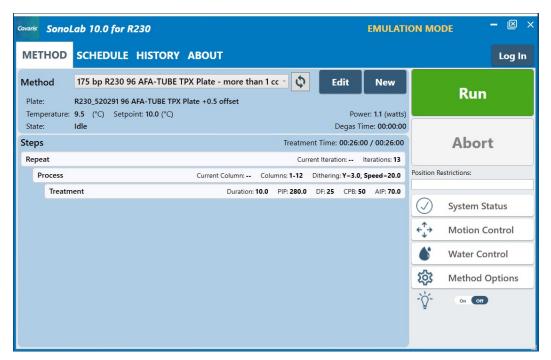


Appendix C: 175 bp Protocol on SonoLab 10.0.0 or Higher for R230 - more than 1 column

Below are some example screenshots for processing of 175 bp protocol with more than 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is not required when processing more than 1 column.

Shearing Protocol in Method Editor



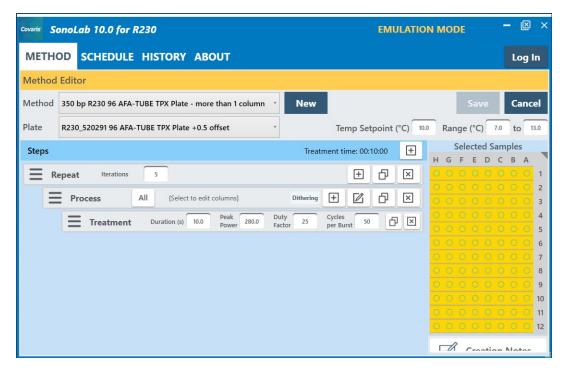


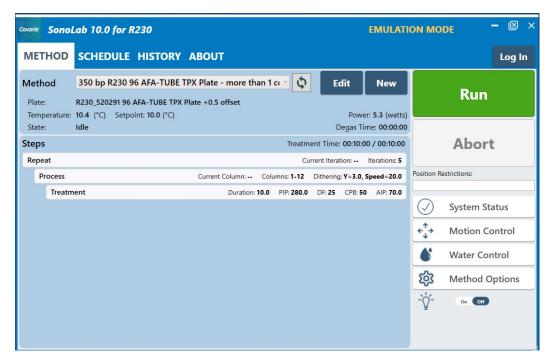


Appendix D: 350 bp Protocol on SonoLab 10.0.0 or Higher for R230 - more than 1 column

Below are some example screenshots for processing of 350 bp protocol with more than 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is not required when processing more than 1 column.

Shearing Protocol in Method Editor



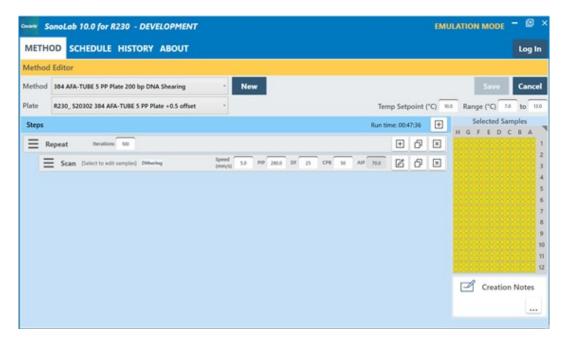


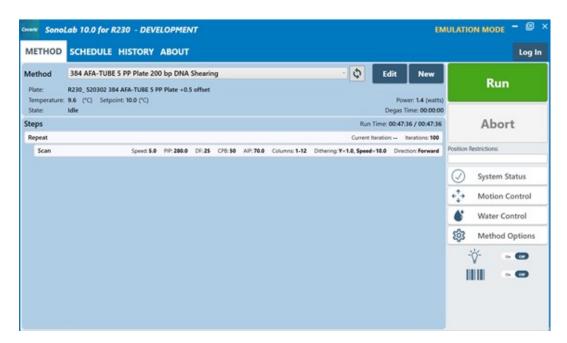


Appendix E: 200 bp Scanning Protocol on SonoLab 10.0.1 or Higher on R230 with 384 AFA-TUBE 5 PP Plate

Below are some example screenshots for processing of 200 bp protocol with 384 AFA-TUBE 5 PP Plate in the Method Editor and Method Screen using the Scan function.

Shearing Protocol in Method Editor







Technical Assistance

Technical Support - Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone
 - United States: Tel: +1 781.932.3959 during the hours of 9:00 a.m. to 4:00 p.m., Monday through Friday, Eastern Standard Time/EST (UTC-05:00)
 - Europe: Tel: 44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Greenwich Mean Time/GMT
- E-mail queries to techsupport@covaris.com or applicationsupport@covaris.com

Revision History

Part Number	Revision	Date	Description of Change
010528	A	7/2020	Initial release
010528	В	6/2021	Addition of 8 AFA-TUBE TPX Strip methods
010528	С	8/2021	Revert to Rev A
010528	D	10/2021	Addition of 384 AFA-TUBE 5 PP Plate method