

Quick Guide

DNA Shearing with R230 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols for the Covaris 96 AFA-TUBE TPX Plate consumable using a Covaris R230 Focused-ultrasonicator instrument.

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 \geq 300 bp: 50 $\mu L,$ \pm 2.5 μL
 - 96 AFA-TUBE TPX Plate for \leq 300 bp: 20 µL, \pm 2.5 µL
- Water Level: +/- 0.5

Sample Guidelines

- DNA Input: 96 AFA-TUBE TPX Plate up to 5 μg 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 AFA-TUBE TPX Plates for long term sample 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.

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 <u>ApplicationSupport@covaris.com</u> 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.

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AFA-TUBE TPX Plate with SonoLab 10.0.0 or Higher

	96 AFA-TUBE TPX	Plate (PN 520291)		
Vessel				
Suggested Sample Volume	5 to	5 to 50 μl		
Rack	Rack R230 96 AFA-TUB	E 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 (#)	Cycles per Burst (#) 50 50			
Delay* Duration (sec)	Delay* Duration (sec) 10 10			
Total Treatment Duration (sec)	130 50			
Sample Volume (µL)	20	50		

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



Appendix A: 175 bp Protocol on SonoLab 10.0 for R230 - 1 column

Following 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.

Shearing Protocol in Method Editor

Covaris Sc	noLab 10.0 for R230 EMULATIO	N MODE	- 🛛 ×
METHO	SCHEDULE HISTORY ABOUT		Log In
Method	Editor		
Method	175 bp R230 96 AFA-TUBE TPX Plate - 1 column Vew	Save	Cancel
Plate	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset * Temp Setpoint (°C)	Range (°C) 7.	0 to 13.0
Steps	Treatment time: 00:04:20 +	Selected Sa	
Ξ Ρι	ocess All [Select to edit columns] Dithering 🛨 🗹 🖸 🗵	00000	0001
Ξ	Repeat Iterations 13 🛨 🗗 🗵	00000	
	Treatment Duration (s) 10.0 Peak 280.0 Puty 25 Cycles 50 D	00000	
	Delay Duration (s): 10.0	00000	0006
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		00000	0 0 0 12
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Covaris Sonol	ab 10.0 for R230 EM	IULATIO	DN MODE - 🗵 🗙
METHOD	SCHEDULE HISTORY ABOUT		Log In
Method Plate: Temperature:	IT5 bp R230 96 AFA-TUBE TPX Plate - 1 column Image: Column and the second	New 5 (watts)	Run
State: Steps Process	Idle Degas Time: C Treatment Time: 00:04:20 / 0 Current Column: Columns: 1 Dithering: Y=3.0, Speec	00:04:20	Abort
Repeat Treatm		tions: 13	Position Restrictions:
Delay	Duratio	on: 10.0	System Status
			← Motion Control
			Water Control
			On Off



Appendix B: 350 bp Protocol on SonoLab 10.0 for R230 - 1 column

Following 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.

Shearing Protocol in Method Editor

Covaris Sc	noLab 10.0 for R230 EMULATIC	N MODE	- 🛛 ×
METHO	D SCHEDULE HISTORY ABOUT		Log In
Method	Editor		
Method	350 bp R230 96 AFA-TUBE TPX Plate - 1 column V New	Save	Cancel
Plate	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset Temp Setpoint (°C)	.0 Range (°C) 7	1.0 to 13.0
Steps	Treatment time: 00:01:40	Selected Sa H G F E D	
	ocess All [Select to edit columns] Dithering 🛨 🖸 🗵	00000	
	Repeat Iterations 5 + D ×	00000	
	Treatment Duration (s) 10.0 Peak 280.0 Duty 25 Cycles 50 D 🖂	00000	
	Delay Duration (s): 10.0	00000	
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		00000	
		00000	0 0 0 12
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Covaris Sonol	ab 10.0 for R230 EMULA	TION MODE 🛛 🗕 🗵 🗙
METHOD	SCHEDULE HISTORY ABOUT	Log In
Method	350 bp R230 96 AFA-TUBE TPX Plate - 1 column Column Edit New	Run
	RES_520251 50 APA-103E 1PX Plate +0.5 diffet 9.8 (°C) Setpoint: 10.0 (°C) Power: 2.6 (wat Idle Degas Time: 00:00:	
Steps Process	Treatment Time: 00:01:40 / 00:01:4 Current Column: Columns: 1 Dithering: Y=3.0, Speed=20.0	ABOIL
Repeat	Current Iterations: Iterations:	Position Restrictions:
Treatm Delay	ent Duration: 10.0 PIP: 280.0 DF: 25 CPB: 50 AIP: 70.0 Duration: 10.0	
		← → Motion Control
		Water Control
		Method Options
		-` <u>\</u> On Off



Appendix C: 175 bp Protocol on SonoLab 10.0 for R230 - more than 1 column

Following 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.

Shearing Protocol in Method Editor

Covaris So	noLab 10.0 for R230 EMULATIO	N MODE	- 🛛 ×
METHO	D SCHEDULE HISTORY ABOUT		Log In
Method	Editor		
Method	175 bp R230 96 AFA-TUBE TPX Plate - more than 1 column V New	Save	Cancel
Plate	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset * Temp Setpoint (°C)	Range (°C) 7.0	to 13.0
Steps	Treatment time: 00:26:00 +	Selected Sar	
	peat Iterations 13		001
Ξ	Process All [Select to edit columns] Dithering +		
	Treatment Duration (s) 10.0 Peak 280.0 Duty 25 Cycles 50 D X		
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		000000	
		000000	
		Creation	Notor

Covaris Sonol	ab 10.0 for R230 EMULA	TION MODE 🛛 🗕 🗵 🗙
METHOD	SCHEDULE HISTORY ABOUT	Log In
Method Plate: Temperature:	Intersection Intersection<	Run
State: Steps Repeat	Idle Degas Time: 00:00: Treatment Time: 00:26:00 / 00:26:0 Current Iteration: Iterations: 1	• Abort
Process Treatm	Current Column: Columns: 1-12 Dithering: Y=3.0, Speed=20. ent Duration: 10.0 PIP: 280.0 DF: 25 CPB: 50 AIP: 70.	
		Water Control
		-ਪੂੱ- on Off



Appendix D: 350 bp Protocol on SonoLab 10.0 for R230 - more than 1 column

Following 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.

Shearing Protocol in Method Editor

Covaris S	noLab 10.0 for R230 EMULATIC	ON MODE 🚽 🗵 🗄
METH	DD SCHEDULE HISTORY ABOUT	Log In
Method	Editor	
Method	350 bp R230 96 AFA-TUBE TPX Plate - more than 1 column V	Save Cancel
Plate	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset Temp Setpoint (°C)	0.0 Range (°C) 7.0 to 13.0
Steps	epeat Iterations 5 Iterations I	Selected Samples H G F E D C B A 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0

Covaris Sonol	ab 10.0 for R230 EMU	ULATIO	n mode 🚽 🖂 🗙
METHOD	SCHEDULE HISTORY ABOUT		Log In
Method	350 bp R230 96 AFA-TUBE TPX Plate - more than 1 ct 🔹 🔯 Edit Ne	ew	Run
	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset 10.4 (°C) Setpoint: 10.0 (°C) Power: 5.3 (n		Kun
State: Steps	Idle Degas Time: 00: Treatment Time: 00:10:00 / 00:		Abort
Repeat Process	Current Iteration: Iteratio Current Column: Columns: 1-12 Dithering: Y=3.0, Speed=		osition Restrictions:
Treatm	ent Duration: 10.0 PIP: 280.0 DF: 25 CPB: 50 AIP:	: 70.0	System Status
			← Motion Control
			Water Control
			Method Options
			-ਊ- On Off



Technical Assistance

- By telephone (+1 781.932.3959) during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at <u>ApplicationSupport@covaris.com</u>

Revision History

Part Number	Revision	Date	Description of Change
010528	А	7/2020	Initial release