

Quick Guide

DNA Shearing with ML230 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols for the Covaris 8 microTUBE-50 V2 Strip, 8 AFA-TUBE® TPX Strip, 8 microTUBE-130 H-Slit V2 Strip, and 8 microTUBE-130 Pre-Slit V2 Strip Consumables using a ML230 Focused-ultrasonicator.

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

- **Temperature:** +/- 3 °C
- **Sample Volume:**
 - microTUBE-50: 55 µL, ± 2.5 µL
 - 8 AFA-TUBE TPX Strip for ≥ 300 bp: 50 µL, ± 2.5 µL
 - 8 AFA-TUBE TPX Strip for ≤ 300 bp: 20 µL, ± 2.5 µL
 - 8 microTUBE-130 H-Slit V2 Strip: 50 µL ± 2.5, 130 µL ± 5
 - 8 microTUBE-130 Pre-Slit V2 Strip: 50 µL ± 2.5, 130 µL ± 5
- **Water Level:** +/- 0.5

Sample Guidelines

- **DNA Input:**
 - microTUBE-50 Strips - up to 5 µg purified DNA
 - 8 AFA-TUBE TPX Strips - up to 5 µg purified DNA
 - microTUBE-130 V2 Strips - 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 microTUBE-50 Strips, microTUBE-130 V2 Strips, or AFA-TUBE TPX Strips 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 associated with them

Instrument Settings

- Recommended settings are subject to change without notice

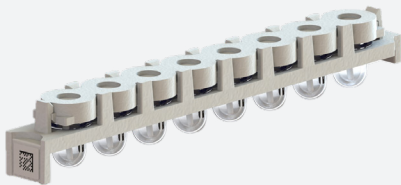
NOTE: DNA fragment representation will vary with analytical systems. Please carry out a time course experiment based on the settings provided in this document to reach the desired fragment size distribution (**Appendix B**).

See www.covaris.com/wp-content/uploads/pn_010522 for updates to this document

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8 microTUBE-50 Strip with SonoLab 10.1.0 or Higher

Vessel	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)* 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)*	
		
Sample Volume	55 µL	
Rack	ML230 Rack 8 microTUBE Strip 50 (PN 500661)	
Plate Definition	ML230_500661 Rack 8 microTUBE Strip 50 +1.8 mm offset	
Dithering	3 mm Y @ 20 mm/s	
Temperature (°C)	12	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	150	350
Repeat/Iterations (#)	30	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (PIP)	350	350
Duty Factor (%)	25	20
Cycles per Burst (#)	1000	1000
Delay Duration (sec)	10	10
Total Treatment Time per Strip (sec)	300	70
Total Run Duration (sec)	600	140

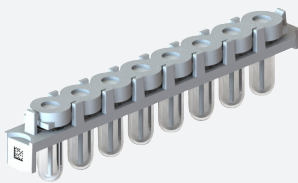
*Both 520174 and 520240 may be used for either the 150 or 350 bp Protocol.

See **Appendix A** for screenshots of protocols in SonoLab 10.1.0 or Higher. See **Appendix B** for how to set up a time course for protocol optimization.

Additional Accessories

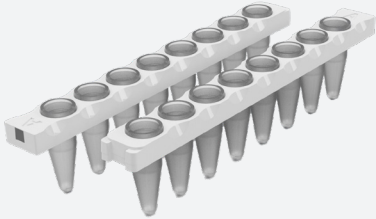
Part Number	Product	Product Description
500541	Centrifuge 8 microTUBE-50 Strip V2 Adapter	Fits the 8 microTUBE-50 Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge

8 microTUBE-130 Strip with SonoLab 10.1.0 or Higher

Vessel	8 microTUBE-130 AFA Fiber Pre-Slit Strip V2 (PN 520217)* 8 microTUBE-130 AFA Fiber H-Slit Strip V2 (PN 520239)*			
				
Rack	ML230 Rack 8 microTUBE Strip (PN 500661)			
Plate Definition	ML230_500661 Rack 8 microTUBE Strip 130 +8 mm offset			
Dithering	1.5 mm Y @ 10 mm/s			
Temperature (°C)	10			
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474			
Base Pair Mode (bp)	150		350	
Volume (µL)	50	130	50	130
Repeat/Iteration (#)	36	48	7	10
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (PIP)	400	400	400	400
Duty Factor (%)	25	25	25	25
Cycles per Burst (#)	600	600	600	600
Delay Duration (sec)	10	10	10	10
Total Treatment Time per Strip (sec)	360	480	70	100
Total Run Duration (sec)	720	960	140	200

*Both 520239 and 520217 may be used for either the 150 or 350 bp Protocol.

8 AFA-TUBE TPX Strip with SonoLab 10.1.0 or Higher

Vessel	8 AFA-TUBE TPX Strip (PN 520292)	
		
Supported Sample Volume*	5 to 50 µL	
Rack	ML230 Rack 8 AFA-TUBE TPX Strip (PN 500660)	
Plate Definition	ML230_500660 Rack 8 AFA-TUBE TPX Strip +12.7 mm offset	
Dithering	3 mm Y @ 20 mm/s	
Temperature (°C)	12	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	16	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	210	210
Duty Factor (%)	25	25
Cycles per Burst (#)	50	50
Delay Duration (sec)	10	10
Total Treatment Duration (sec)	160	70
Total Run Duration (sec)	320	140
Sample Volume (µL)	20	50

*Current protocols support 20 µL sample volume for 175 bp and 50 µL sample volume for 350 bp.

See **Appendix A** for screenshots of a programmed protocol in SonoLab 10.1.0. The 8 AFA-TUBE TPX Strip will follow the protocol order for the 8 microTUBE-50: Process, Repeat, Delay, and Treatment. See **Appendix B** for how to set up a time course for protocol optimization.

Appendix A: Protocol on SonoLab 10.1.0 or Higher

Programming an Acoustic Method for the ML230 Focused-ultrasonicator.

1. After instrument initiation and homing click on “New” which brings you to the “Method Editor”.
2. Select The appropriate plate for the tubes used in the method from the “Plate Definition” drop-down list (such as ML230_500660 Rack 8 AFA-TUBE TPX Strip + 12.7 mm offset).
3. In the “Step” section click the “+” sign. For example: Process.
4. Click on the “+” sign on the Process line, then select repeat and select how many times to repeat, then OK.
5. Click the “edit/pencil” button on the Process line to set Dithering parameters.
6. Click on the “+” sign in the “Process line and select “Delay” fill in time (sec).
7. Click on the “+” sign and select “Treatment”, fill in “Duration”, “Peak Incident Power”, “Duty Factor”, and “Cycles per Burst”.
8. Go to the top section and fill in temperature °C.

Following are some example screenshots of the 150 bp protocol for the microTUBE-50 in the Method Editor and Method Screen.

NOTE: Repeat (“Iterations”) is programmed after the Process.

SonoLab 10.1 for ML230 **EMULATION MODE** ×

METHOD SCHEDULE HISTORY ABOUT Log In

Method Editor

Method: 150 bp ML230 microTUBE-50 Strip V2 New Save Cancel

Plate: ML230_500661 Rack 8 microTUBE Strip 50 +1.8 mm offset Temp Setpoint (°C) 12.0 Range (°C) 9.0 to 15.0

Steps Treatment time: 00:10:00 +

Process Dithering + ✎ 📄 ✕

Repeat Iteration 30 + 📄 ✕

Delay Duration (s): 10.0 📄 ✕

Treatment Duration (s) 10.0 Peak Power 350.0 Duty Factor 25 Cycles per Burst 1000 Avg Power 87.5 📄 ✕

Shearing Protocol in Method Editor

SonoLab 10.1 for ML230 **EMULATION MODE** ×

METHOD SCHEDULE HISTORY ABOUT Log In

Method: 150 bp ML230 microTUBE-50 Strip V2 ↺ Edit New Run

Plate: ML230_500661 Rack 8 microTUBE Strip 50 +1.8 mm offset

Temperature: 12.0 (°C) Setpoint: 12.0 (°C) Power: 3.7 (watts)

State: Idle Degas Time: 00:00:00

Steps Treatment Time: 00:10:00 / 00:10:00

Process Dithering: Y=3.0, Speed=20.0

Repeat Current Iteration: -- Iterations: 30

Delay Duration: 10.0

Treatment Duration: 10.0 PIP: 350.0 DF: 25 CPB: 1000 AIP: 87.5

Abort

✓ System Status

↕ Motion Control

💧 Water Control

⚙️ Method Options

💡 On Off

Shearing Protocol in Method Screen

Appendix B: Time Course – Create Pulsing Method using SonoLab 10.1.0 or Higher

Refer to the ML230 User Manual for detailed instructions on pulsing method creation.

www.covaris.com/wp-content/uploads/pn_010516.pdf

1. **Time Course:** A time course is necessary to optimize shearing protocols based on user needs. Covaris recommends a total of 6 time points for any target size, using the maximum number of samples (N = 8 for strip consumables) for each time point in the time course. The time course shall be performed by starting with the nearest recommended protocol (containing “Z” iterations) and increasing or decreasing the total number of iterations used to adjust total treatment time.
2. **Time Course Set-Up:**
 - a) Fill Covaris Consumable with appropriate volume of lambda or genomic DNA in a desired buffer at a concentration of 10 ng/μL.
 - Recommended buffer: Tris-EDTA
 - b) Input protocol settings into SonoLab with a value of Z-2 or Z-4 iterations to begin: Z value is as published in the shearing protocols. The choice between Z-2 or Z-4 iterations for the first time point is determined based on target size.
 - I.e. For methods shearing to above 200 bp, # iterations for first time point should be Z-2.
 - I.e. For methods shearing to below 200 bp, # iterations for first time point should be Z-4.
 - c) Insert tubes into the ML230 and run protocol.
 - d) Once the treatment is complete, remove samples from the Instrument.
 - e) Transfer samples to a LoBind™ tube for sizing analysis.
 - f) Repeat Steps ‘a’ through ‘e’ until a total of 6 time points have been tested. For methods shearing to above 200 bp, the iterations should change in ‘+1’ increments, whereas for methods shearing to below 200 bp, the iterations should change in ‘+2’ increments.
 - I.e. For methods shearing to above 200 bp, # iterations for 6 runs should be Z-2, Z-1, Z, Z+1, Z+2, Z+3.
 - I.e. For methods shearing to below 200 bp, # iterations for 6 runs should be Z-4, Z-2, Z, Z+2, Z+4, Z+6.
 - g) If extra time points are necessary, additional runs can be performed by adjusting the # iterations.
 - h) Retain total iterations or duration(s) used to obtain desired fragment sizes for programming into SonoLab and future use.

NOTE: Do not alter settings when running a time course. Only iterations should be altered.

DNA Shearing Time Course Examples*

Note:

- Sample for each time point - Appropriate volume of lambda or genomic DNA (10 ng/μL) in Tris-EDTA Buffer.
- Only settings highlighted in '**red**' should change.
- Final setting to be determined based on time course results.

8 microTUBE-50 AFA Fiber Strip V2 150 bp Protocol Time Course Example Setup

Timepoint	1	2	3	4	5	6
Test Strip	1	2	3	4	5	6
# Iterations	Z - 4	Z - 2	Z	Z + 2	Z + 4	Z + 6
Instrument Settings on ML230						
Repeat / Iterations (#)	26	28	30	32	34	36
Repeat Process Treatment Duration (sec)	10	10	10	10	10	10
Peak Incident Power (W)	350	350	350	350	350	350
Duty Factor (%)	25	25	25	25	25	25
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000
Delay Duration (sec)	10	10	10	10	10	10
Total Treatment Duration (sec)	260	280	300	320	340	360
Total Run Duration (sec)	520	560	600	640	680	720
Volume (μL)	55	55	55	55	55	55
Dithering	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s
Temperature (°C)	12	12	12	12	12	12

8 microTUBE-50 AFA Fiber Strip V2 350 bp Protocol Time Course Example Setup

Timepoint	1	2	3	4	5	6
Test Strip	1	2	3	4	5	6
# Iterations	Z - 2	Z - 1	Z	Z + 1	Z + 2	Z + 3
Instrument Settings on ML230						
Repeat / Iterations (#)	5	6	7	8	9	10
Repeat Process Treatment Duration (sec)	10	10	10	10	10	10
Peak Incident Power (W)	350	350	350	350	350	350
Duty Factor (%)	25	25	25	25	25	25
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000
Delay Duration (sec)	10	10	10	10	10	10
Total Treatment Duration (sec)	50	60	70	80	90	100
Total Run Duration (sec)	100	120	140	160	180	200
Volume (μL)	55	55	55	55	55	55
Dithering	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s
Temperature (°C)	12	12	12	12	12	12

8 AFA-TUBE TPX Strip 175 bp Protocol Time Course Example Setup

Timepoint	1	2	3	4	5
Test Strip	1	2	3	4	5
# Iterations	Z - 4	Z - 2	Z	Z + 2	Z + 4
Programmed Conditions					
Repeat / Iterations (#)	12	14	16	18	20
Repeat Process Treatment Duration (sec)	10	10	10	10	10
Peak Incident Power (W)	210	210	210	210	210
Duty Factor (%)	25	25	25	25	25
Cycles per Burst (#)	50	50	50	50	50
Delay Duration (sec)	10	10	10	10	10
Total Treatment Duration (sec)	120	140	160	180	200
Total Run Duration (sec)	240	280	320	360	400
Volume (µL)	20	20	20	20	20
Dithering	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s
Temperature (°C)	12	12	12	12	12

8 AFA-TUBE TPX Strip 350 bp Protocol Time Course Example Setup

Timepoint	1	2	3	4	5
Test Strip	1	2	3	4	5
# Iterations	Z - 2	Z - 1	Z	Z + 1	Z + 2
Programmed Conditions					
Repeat / Iterations (#)	5	6	7	8	9
Repeat Process Treatment Duration (sec)	10	10	10	10	10
Peak Incident Power (W)	210	210	210	210	210
Duty Factor (%)	25	25	25	25	25
Cycles per Burst (#)	50	50	50	50	50
Delay Duration (sec)	10	10	10	10	10
Total Treatment Duration (sec)	50	60	70	80	90
Total Run Duration (sec)	100	120	140	160	180
Volume (µL)	50	50	50	50	50
Dithering	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s	3 mm Y @ 20 mm/s
Temperature (°C)	12	12	12	12	12

*Time Course examples adhere to and maintain consistency of constraints listed on consumable outline pages (i.e. Dithering, Temperature).

Support and Technical Assistance

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

- **Telephone:**
 - US & APAC: +1 781.932.3959, during the hours of 8:30 a.m. to 5:00 p.m. (EST), Monday through Friday
 - EU: +44 (0)845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. (GMT), Monday through Friday
- **E-mail:**
 - US Customer Service: customerservice@covaris.com
 - EU/UK Customer Service: emeacustomerservice@covaris.com
 - APAC Customer Service: APACcustomerservice.@covaris.com
 - Service and Instrumentation: techsupport@covaris.com
 - Solutions: applicationsupport@covaris.com

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