

Quick Guide:

truSHEAR Buffer DNA Shearing

This Quick Guide provides DNA Shearing protocols when using the truSHEAR Buffer (PN 520247 and PN 520248) and a Covaris Focused-ultrasonicator. This guide contains protocols for shearing DNA to 150 and 200bp in microTUBE-50 and microTUBE-130 consumables, on the M220, ME220, S220, E220, and LE220.

Revision History

Part Number	Revision	Date	Description of change
010401	A	5/2017	Protocols for microTUBE-50, 150bp and 200bp
010401	B	10/2018	Protocols for microTUBE-130, 150bp and 200bp

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

- Temperature +/-2°C
- Sample volume
 - o microTUBE-50: 55 µl, +/- 2.5 µl
 - o microTUBE-130: 130 µl +/- 5 µl
- Water Level +/- 1

Sample preparation guidelines

Consumable	Total Shearing Volume	DNA Sample Volume	truSHEAR Buffer Volume
microTUBE-50	55 µl	50 µl	5 µl
microTUBE-130	132 µl	120 µl	12 µl

- **DNA input:** protocols are optimized for low concentrations of pure DNA up to 10ng/µl
- **DNA quality: purified genomic DNA.** For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.
Note, truSHEAR Buffer increases yield relative to TE or low TE when shearing a purified substrate.
- **DO NOT use the microTUBE for storage. Samples should be transferred after processing.**
- Product User Manual: https://covaris.com/wp-content/uploads/pn_010400.pdf

Instrument setup

- Refer to the instrument manuals for complete setup.
- microTUBEs have specific racks associated with them.
- The water level is set on the RUN scale in the Start position.
- E220 and LE220 protocols may require X and/or Y-dithering. Refer to Appendices A and B for instructions.

Instrument settings



- Recommended settings are subject to change without notice.
- Settings developed without truSHEAR buffer maybe affected, please refer to this document for guidelines
- Mean DNA fragment size distributions are based on smear analysis (25-700bp) of fragments generated on a Fragment Analyzer (AATI) using the High Sensitivity 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 hyperlink for updates to this document.

http://covarisinc.com/wp-content/uploads/pn_010401.pdf


M220 Focused-ultrasonicator

Vessel	microTUBE-50 AFA Fiber Screw-Cap (PN 520166)		Vessel	microTUBE-130 AFA Fiber Snap-Cap (PN 520045)	
					
Holder	XTU 500414		Holder	XTU 500414	
Insert	PN 500488		Insert	PN 500489	
Sample Volume	55 μ l		Sample Volume	132 μ l	
Water Temperature	20°C		Water Temperature	20°C	
Target BP (Smear)	150	200	Target BP (Smear)	150	200
Peak Incident Power (W)	75	75	Peak Incident Power (W)	50	50
Duty Factor	10%	10%	Duty Factor	20%	20%
Cycles per Burst	1000	1000	Cycles per Burst	200	200
Treatment Time (s)	450	220	Treatment Time (s)	350	170





Even if the Water Level check button is green in SonoLab, please check that water is in contact with Insert when using microTUBE-50 in the M220.





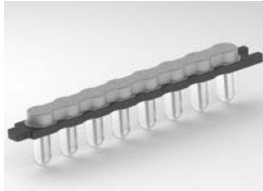
S220 Focused-ultrasonicator

Vessel	microTUBE-50 AFA Fiber Screw-Cap (PN 520166)	
		
Holder	500492	
Sample Volume	55 μ l	
Water Level	10	
Water Temperature	7°C	
Target BP (Smear)	150	200
Peak Incident Power (W)	100	75
Duty Factor	30%	20%
Cycles per Burst	1000	1000
Treatment Time (s)	145	105




ME220 Focused-ultrasonicator: microTUBE-50

<p>Vessel</p>	<p>microTUBE-50 AFA Fiber Screw-Cap (PN 520166)</p> 	<p>8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)</p> <p>8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)</p> 
<p>Rack</p>	<p>Rack 4-place microTUBE Screw-Cap PN 500522</p>	<p>Rack 8 microTUBE Strip V2 PN 500518</p>
<p>Waveguide</p>	<p>PN 500534</p>	<p>PN 500526</p>
<p>Sample Volume</p>	<p>55 μl</p>	<p>55 μl</p>
<p>Water Level</p>	<p>5.5</p>	<p>5.5</p>
<p>Water Temperature</p>	<p>9°C</p>	<p>9°C</p>
<p>Target BP (Smear)</p>	<p>150 200</p>	<p>150 200</p>
<p>Duration (s)</p>	<p>170 78</p>	<p>280 125</p>
<p>Peak Power (W)</p>	<p>75 75</p>	<p>50 50</p>
<p>Duty Factor (%)</p>	<p>25% 25%</p>	<p>30% 30%</p>
<p>Cycles per Burst</p>	<p>1000 1000</p>	<p>1000 1000</p>

ME220 Focused-ultrasonicator: microTUBE-130

Vessel	microTUBE-130 AFA Fiber Screw-Cap (PN 520216) 	8 microTUBE-130 AFA Fiber Strip V2 (PN 520217) 8 microTUBE-130 AFA Fiber H Slit Strip V2 (PN 520239) 	
Rack	Rack 4-place microTUBE Screw-Cap PN 500522	Rack 8 microTUBE Strip V2 PN 500518	
Waveguide	PN 500534	PN 500526	
Sample Volume	132 μ l	132 μ l	
Water Level	9	9	
Water Temperature	20°C	20°C	
Target BP (Smear)	150 200	150 200	
Duration (s)	240 145	250 160	
Peak Power (W)	75 70	75 70	
Duty Factor (%)	25% 20%	25% 20%	
Cycles per Burst	1000 1000	1000 1000	
Vessel	microTUBE AFA Fiber Pre-Slit Snap-Cap (PN 520045) 	microTUBE AFA Fiber Crimp-Cap (PN 520052) 	8 microTUBE Strip V1 (PN 520053) 
Rack	Rack - Snap-Cap/Crimp-Cap/8 microTUBE Strip V1 PN 500514		
Waveguide	PN 500526		
Sample Volume	130 μ l		
Water Level	6	7	6
Water Temperature	20°C	20°C	20°C
Target BP (Smear)	150 200	150 200	150 200
Duration (s)	265 165	280 165	247 175
Peak Power (W)	75 70	75 70	75 70
Duty Factor (%)	25 20	25 20	25 20
Cycles per Burst	1000 1000	1000 1000	1000 1000





E220 Focused-ultrasonicator: microTUBE-50

	Vessel	microTUBE-50 Screw-Cap (PN 520166) 	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174) 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240) 	96 microTUBE-50 AFA Fiber Plate (PN 520168) 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232) 	
	Sample Volume	55 µl			
E220	Racks	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip (PN 500444)	No Rack needed	
	Plate Definitions	“E220_500308 Rack 24 Place microTUBE-50 Screw-Cap +6.5mm offset”	“E220_500444 Rack 12 Place 8 microTUBE-50 Strip V2 -10mm offset”	“E220_520168 96 microTUBE-50 Plate - 10.5mm offset”	
E220 evolution	Racks	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack E220e 8 microTUBE Strip V2 (PN 500437)	Non-Compatible	
	Plate Definitions	“500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset”	“500437 E220e 8 microTUBE-50 Strip V2 - 10mm offset”	N/A	
All	Temperature (°C)	7			
	Water Level	6	-2	0	
	Intensifier (PN 500141)	Yes	Yes	Yes	
	Y-dithering	No	No	Yes 0.5mm Y-dither at 10mm/s	
	Target BP (Smear)	150	200	150	200
	Peak Incident Power (W)	100	75	75	75
	Duty Factor	30%	20%	15%	15%
	Cycles per Burst	1000	1000	500	500
	Treatment Time (s)	145	105	400	255
				360	180


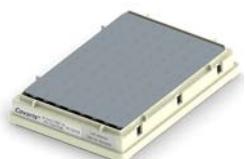


The Y-dithering function is required for shearing with 96 microTUBE-50 plate (PN 520168). This function is only available on SonoLab versions 7.3 and up. Please see Appendix A for detailed instructions.

E220 Focused-ultrasonicator: microTUBE-130

	Vessel	microTUBE AFA Fiber Snap-Cap (PN 520045) 	microTUBE AFA Fiber Crimp-Cap (PN 520052) 	8 microTUBE Strip (PN 520053) 	96 microTUBE Plate (PN 520078) 96 microTUBE AFA Fiber Plate Thin Foil (PN 520230) 				
	Sample Volume	130 µl							
E220	Racks	Rack 24 Place microTUBE Snap-Cap (PN 500111)	Rack 96 Place microTUBE Crimp-Cap (PN 500282)	Rack 12 Place 8 microTUBE Strip (PN 500191)	No Rack needed				
	Plate Definitions	"500111 24 microTUBE snap +4mm offset"	"E220_500282 Rack 96 Place microTUBE -6mm offset"	"E220_500191 8 microTUBE strip Plate -6mm offset"	"E220_520078 96 microTUBE Plate -6mm offset" "E220_520230 96 microTUBE Plate Thin Foil - 6mm offset"				
	Water Level	6							
	Intensifier (PN 500141)	Yes							
	Y-dithering	No							
E220 evolution	Racks	Rack E220e 8 Place microTUBE Crimp and Snap Cap (PN 500433)		Rack E220e 8 microTUBE Strip (PN 500430)	Non-Compatible				
	Plate Definitions	"500433 E220e 8 microTUBE Crimp and Snap Cap -3.7mm offset"		"500430 E220e 8 microTUBE Strip -6mm offset"	N/A				
	Water Level	6							
	Intensifier (PN 500141)	Yes							
	Y-dithering	No							
All	Temperature (°C)	7							
	Target BP (Peak)	150	200	150	200	150	200	150	200
	Peak Incident Power (W)	175	175	175	175	175	175	175	175
	Duty Factor	10%	10%	10%	10%	15%	10%	15%	15%
	Cycles per Burst	200	200	200	200	1000	200	200	200
	Treatment Time (s)	455	205	470	210	355	255	350	150

LE220 Focused-ultrasonicator: microTUBE-50




Vessel	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174) 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240) 	96 microTUBE-50 AFA Fiber Plate (PN 520168) 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232) 
	Sample Volume	55 µl

LE220	Racks	Rack – XT 12 Place 8 microTUBE Strip V2 (PN 500485)	No Rack needed		
	Plate Definitions	“LE220_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset”	“LE220_520168 96 microTUBE-50 Plate -12mm offset”		
	Water Level	-2			
	X and/or Y-dithering	Yes 0.5mm X-dither & 0.5mm Y-dither at 10mm/sec			
	Temperature (°C)	7			
	Target BP (Smear)	150	200	150	200
	Peak Incident Power (W)	450	450	450	450
	Duty Factor	20%	20%	20%	20%
	Cycles per Burst	1000	1000	1000	1000
	Treatment Time (s)	360	160	540	230



The X-dithering and Y-dithering functions are both required for shearing with the 8 microTUBE-50 AFA Fiber Strip V2 and the 96 microTUBE-50 AFA Fiber Plate. These functions are only available on SonoLab versions 7.3 and up. Please see Appendix B for detailed instructions.

LE220 Focused-ultrasonicator: microTUBE-130

	Vessel	microTUBE AFA Fiber Crimp-Cap (PN 520052) 	8 microTUBE Strip V1 (PN 520053) 	96 microTUBE Plate (PN 520078) 96 microTUBE AFA Fiber Plate Thin Foil (PN 520230) 			
	Sample Volume	130 µl					
LE220	Racks	Rack 96 Place microTUBE Crimp-Cap (PN 500282)	Rack 12 Place 8 microTUBE Strip (PN 500191)	No Rack needed			
	Plate Definitions	"LE220_500282 Rack 96 Place microTUBE -4mm offset"	"LE220_500191 Rack 8 microTUBE Strip -4mm offset"	"LE220_520078 96 microTUBE Plate -4mm offset" "LE220_520230 96 microTUBE Plate Thin Foil -4mm offset"			
	Water Level	6					
	X and/or Y-dithering	No					
	Temperature (°C)	7					
	Target BP (Smear)	150	200	150	200	150	200
	Peak Incident Power (W)	450	450	450	450	450	450
	Duty Factor	30%	30%	30%	30%	30%	30%
	Cycles per Burst	200	200	200	200	200	1000
	Treatment Time (s)	440	205	450	195	490	220

Additional Accessories

	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
Centrifuge 8 microTUBE Strip V2 Adapter	Fits the 8 microTUBE Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge	500541

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

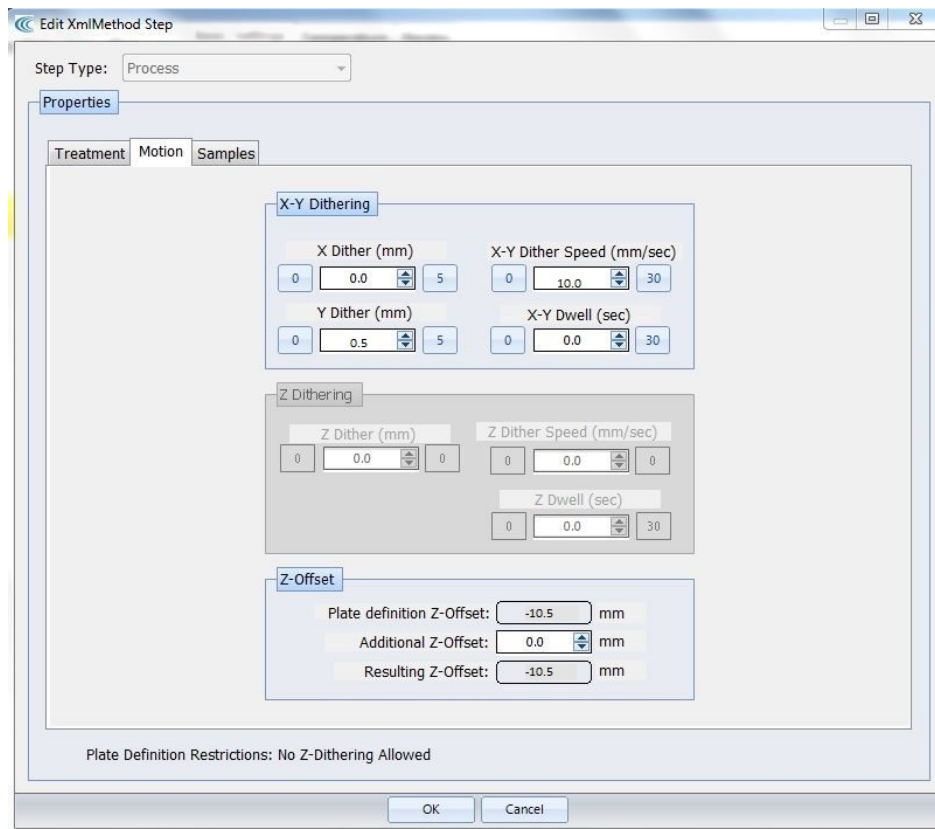
Appendix A – Using Y-dithering with SonoLab 7.3 and up

A Y-dithering step is required for DNA shearing with the 96 microTUBE-50 Plate

- This feature is only available on SonoLab versions 7.3 and up.
- To obtain a copy of the SonoLab 7.3 and the Plate Definition installers, please employ the Registered Users Login on the Covaris website, www.covarisinc.com
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at TechSupport@covarisinc.com.

Use the following steps to include Y-dithering in sample treatment

1. Go into the Method Editor
2. Select 'Add Step' and enter the treatment settings for the desired fragment size
 - a. **Note:** The following steps must be done for each individual treatment
3. Select the Motion tab
4. Enter the following values into the 'X-Y Dithering Box'
 - a. Y Dither (mm): **0.5**
 - b. X-Y Dither Speed (mm/sec): **10.0**
 - c. Both X Dither (mm) and X-Y Dwell (sec) should be set to **0**



Appendix B – Using X and Y-dithering with SonoLab 7.3 and up

X and Y-dithering are required for DNA shearing with the 8 microTUBE-50 AFA Fiber Strip V2 and microTUBE-50 AFA Fiber Plate

96

- This feature is only available on SonoLab versions 7.3 and up.
- There are dithering limitations on instruments with serial numbers below 2000.
- To obtain a copy of the SonoLab 7.3 and the Plate Definition installers, please employ the Registered Users Login on the Covaris website, www.covarisinc.com
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at TechSupport@covarisinc.com.

Use the following steps to include X-dithering and Y-dithering in sample treatment:

1. Go into the Method Editor
2. Select 'Add Step' and enter the treatment settings for the desired fragment size
 - a. **Note:** The following steps must be done for each individual treatment
3. Select the Motion tab
4. Enter the following values into the 'X-Y Dithering' box
 - a. X Dither (mm): **0.5**
 - b. Y Dither (mm): **0.5**
 - c. X-Y Dither Speed (mm/sec): **10.0**
 - d. X-Y Dwell (sec) should be set to **0**

