



**Covaris DNA Shearing Verification Kit for
AFA-TUBE® TPX Plate on the R230**

(PN 520120)

Introduction

This kit ([PN 520120](#)) allows users to verify the performance of their Covaris Focused-ultrasonicator using the 96 AFA-TUBE TPX Plate on the R230. The kit may be used for periodic assurance of performance, for instrument QC, or employed in troubleshooting when applications perform differently than expected. The kit contains a pre-fragmented Reference Sample of Lambda DNA, as well as unfragmented Test Sample of Lambda DNA sufficient for performance testing. Covaris recommends diluting both the Test Sample and the Reference Sample 1:3 in Tris-EDTA (TE) buffer and performing the analysis on a high sensitivity DNA analysis kit. Simply shear the Test Sample DNA with the R230 and compare the results to the Reference Sample using the Agilent® Fragment Analyzer (or equivalent).

Kit Contents

This kit includes:

- Reference Sample (**Blue Cap**): 40 µL of pre-fragmented DNA with an average fragment size distribution between 150 & 300 bp.
- Test Sample (**Red Cap**): two tubes each containing 1,100 µL of Lambda DNA. SDS information is available at: http://covaris.com/wp-content/uploads/pn_010379.pdf

NOTE: Check the lowest and highest allowed DNA concentration of your DNA analyzer prior to shearing and performing DNA distribution analysis.

Customer Supplied Materials

- Fragment Analysis Reagents (Agilent Bioanalyzer High Sensitivity DNA Kit cat# 5067-4626, Agilent Fragment Analyzer High Sensitivity NGS Fragment Kit cat# DNF-474, or equivalent)
- R230 Focused-ultrasonicator ([PN 500620](#))
- 96 AFA-TUBE TPX Plate ([PN 520291](#))
- Rack 96 AFA-TUBE TPX ([PN 500668](#))
- 8 microTUBE Strip Foil Seal ([PN 520108](#)) or 96 microTUBE Plate Thin Foil Seals (25) ([PN 520235](#))
- 1x Tris-EDTA, pH 8.0 (TE buffer, for dilution of samples)

Storage

- 1 year at 2–8 °C

Workflow

- We recommend diluting the Test Sample 1:3 with TE Buffer for a DNA concentration of around 8–10 ng/µL. When diluted, this kit may be used to perform verification tests with up to 36 filled columns (n=288 samples, or 3 AFA-TUBE TPX plates) with the 1:3 DNA solution.
- Dilute the Test Sample (**Red Cap**) 1:3 in TE buffer.
 - Add 2,000 µl TE buffer to an appropriately sized vial (holds >3.0 mL). Transfer 1,000 µL of the Test Sample to the vial containing 2,000 µl TE buffer. Mix well. (Note: This can be done for both Test Sample vials or portions of the Test Sample vials, keeping the 1:3 ratio.)
- Dilute the Reference Sample (**Blue Cap**) 1:3 in TE Buffer before analysis. (For example, take 15 µL of the Reference Sample and add it to 30 µL of TE Buffer) Mix well. This will be used during the Analysis steps.

- Fill 20 µL of the 1:3 diluted Test Sample into the 96 AFA-TUBE TPX Plate ([PN 520291](#)). We recommend running at least 2 columns of samples for each verification run.
- Quick spin the 96 AFA-TUBE TPX plate (about 10 seconds in a swinging bucket centrifuge).
- Place the 96 AFA-TUBE TPX plate in the Rack 96 AFA-TUBE TPX Plate (R230 Rack [PN 500668](#)).
- Process these samples following instrument settings given in **Table 1** for the R230.
- Quick spin the 96 AFA-TUBE TPX plate (about 10 seconds in a swinging bucket centrifuge).
- Analyze these samples alongside at least 3 replicates of the 1:3 diluted Reference.

NOTE: The Reference Sample is already fragmented and does not need to be further processed.

Instrument Parameters/Settings

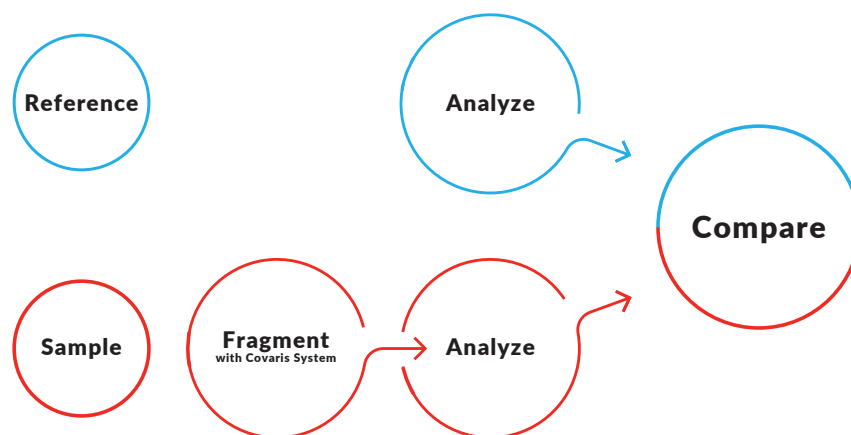
This kit is compatible with the 96 AFA-TUBE TPX plate ([PN 520291](#)). Please follow the settings carefully for the R230 Focused-ultrasonicator and the 96 AFA-TUBE TPX Plate. See **Appendix C** for a screenshot of the protocol in SonoLab 10.

Instrument	AFA-TUBE	Rack	Plate Definition	Temp.	Sample Volume	PIP	Duty Factor	Cycles per Burst	Dithering	Time	Delay Time*	Repeats
R230	520291	500668	"R230_520291 96 AFA-TUBE TPX Plate +0.5 offset.plt"	10 °C	20 µL	280 W	25%	50	3 mm y-dither at 20 mm/s	10 sec	10 sec	11

Table 1. Covaris Instrument DNA Shearing Settings for R230 (see **Appendix C**). *Delay time can be programmed if running less than 1 column (not recommended; see **Appendix C**).

Analysis

- Analyze the fragment size distribution of both Reference and Processed Test Samples in the same analytical run. Load at least 3 replicates of the Reference Sample.
- Compare fragment size distributions to verify that the Covaris Focused-ultrasonicator is performing correctly.



Interpretation

For analysis, employ the available analysis device (Agilent Bioanalyzer 2100, Agilent Fragment Analyzer, Perkin Elmer® LabChip®, Agilent 2200 TapeStation, Bio-Rad® Experion®, Agarose gel, or equivalent). **It is important to run both the Reference and Processed Test Samples on the same chip or gel to normalize the results from analytical assay variations.**

For each sample, determine the average size of the fragment distribution using a range of 50–900 bp.

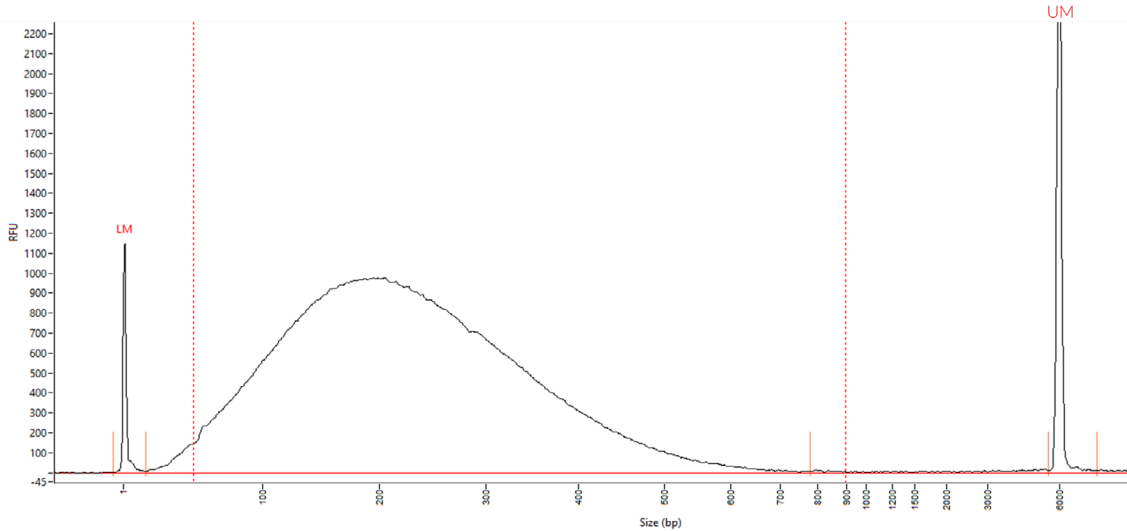


Figure 1. Smear analysis range 50–900 bp (Avg. size 220 bp) using ProSize 3.0.

For the Reference Sample replicates (minimum of 3), calculate the average and the Coefficient of Variation. For the Processed Test Samples (minimum of 16), calculate the average and the Coefficient of Variation. Compare the calculated average sizes of the Reference and Processed Test Samples using **Table 2**.

	Interpretation
Coefficient of Variation of Processed Samples < 10%	Covaris system OK
Coefficient of Variation of Processed Samples > 10%	Contact Covaris
Coefficient of Variation of Reference Samples < 10%	Covaris system OK
Coefficient of Variation of Reference Samples > 10%	Contact Covaris
Reference Sample in the 100–300 bp Range	Covaris system OK
Reference Sample out of the 100–300 bp Range	Problem with fragment size distribution analysis
Processed Test Samples within 10% of the Reference Sample	Covaris system OK
Processed Test Samples not within 10% of the Reference Sample	Contact Covaris

Table 2. Covaris Performance Verification Kit interpretation. Covaris Contact: applicationsupport@covaris.com.

Appendix A:

To compare the Processed Test Sample results to the Reference Sample, please use this equation:

$$\% \text{ of Reference Sample} = 100 - \left(100 \times \frac{\text{Average value for Processed Test Samples}}{\text{Average value for Reference Samples}} \right)$$

Appendix B: Troubleshooting

- If the Coefficient of Variation of the sixteen Processed Test Samples is > 10% or if the average fragment size is > 10% different from the Reference Sample, contact Covaris at applicationsupport@covaris.com.
- When contacting Covaris, please include the following information:
 - Instrument serial number
 - Lot number of the 96 AFA-TUBE TPX plate
 - Lot number of the DNA Shearing Verification Kit ([PN 520120](#))
 - Electropherogram profiles (.pdf and/or raw data files)
 - Picture of 96 AFA-TUBE TPX plate in the START position, highlighting the water level on the side of the plate.

Appendix C: Screenshot of R230 DNA Shearing Verification Test Method

The screenshot shows the 'METHOD' tab for the 'R230 DNA Shearing Verification Kit Protocol'. The interface includes tabs for 'METHOD', 'SCHEDULE', 'HISTORY', 'ADMIN', and 'ABOUT'. The method name is 'R230 DNA Shearing Verification Kit Protocol' with a refresh icon and 'Edit' and 'New' buttons. Parameters listed are: Plate: R230_520291 96 AFA-TUBE TPX Plate +0.5 offset; Temperature: 10.3 (°C) Setpoint: 10.0 (°C); Power: 5.0 (watts); State: Idle; Degas Time: 00:03:28. The 'Steps' section shows a 'Repeat' step with 'Current Iteration: --' and 'Iterations: 11'. Below it is a 'Process' step with 'Current Column: --', 'Columns: 1-2', 'Dithering: Y=3.0, Speed=20.0'. At the bottom is a 'Treatment' step with 'Duration: 10.0', 'PIP: 280.0', 'DF: 25', 'CPB: 50', and 'AIP: 70.0'. The 'Treatment Time' is 00:03:40 / 00:03:40.

Figure 1. Recommended method for 2 or more columns programmed for the DNA shearing verification kit in SonoLab 10.0 with the 96 AFA-TUBE TPX Plate ([PN 520291](#)).

The screenshot shows the 'METHOD' tab for the 'R230 DNA Shearing Verification Kit Protocol - 1 column'. The interface includes tabs for 'METHOD', 'SCHEDULE', 'HISTORY', 'ADMIN', and 'ABOUT'. The method name is 'R230 DNA Shearing Verification Kit Protocol - 1 column' with a refresh icon and 'Edit' and 'New' buttons. Parameters listed are: Plate: R230_520291 96 AFA-TUBE TPX Plate +0.5 offset; Temperature: 10.4 (°C) Setpoint: 10.0 (°C); Power: 5.4 (watts); State: Idle; Degas Time: 00:09:12. The 'Steps' section shows a 'Process' step with 'Current Column: --', 'Columns: 1', 'Dithering: Y=3.0, Speed=20.0'. Below it is a 'Repeat' step with 'Current Iteration: --' and 'Iterations: 11'. At the bottom is a 'Treatment' step with 'Duration: 10.0', 'PIP: 280.0', 'DF: 25', 'CPB: 50', and 'AIP: 70.0'. Below the 'Treatment' step is a 'Delay' step with 'Duration: 10.0'. The 'Treatment Time' is 00:03:40 / 00:03:40.

Figure 2. Method for 1 column programmed for the DNA shearing verification kit in SonoLab 10.0 with the 96 AFA-TUBE TPX Plate ([PN 520291](#)).