

User Manual

Covaris DNA Shearing Verification Kit for AFA-TUBE TPX Consumables

(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 or 8 AFA-TUBE TPX Strip. 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. Alternatively, the undiluted solutions can be used in combination with a standard sensitivity DNA analysis kit. Simply shear the Test Sample DNA with your Covaris instrument and compare the results to the Reference Sample using the Agilent® Bioanalyzer 2100 (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 1100 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.

- The kit contains enough undiluted material to perform verification tests with up to 5 filled columns (n=40 samples) of 50 µL samples of the Test Sample DNA solution at 30 ng/µL.
- We recommend diluting the Test Sample 1:3 with TE Buffer for a DNA concentration of 10 ng/µL. When diluted, this kit may be used to perform verification tests with up to 15 filled columns (n=120 samples) with the 1:3 DNA solution at 10 ng/µL.

Customer Supplied Materials

- Fragment Analysis Reagents (Agilent Bioanalyzer High Sensitivity DNA Kit PN 5067-4626 or equivalent)
- Focused-ultrasonicator: see Instrument Tables for settings
- AFA-TUBE TPX from the respective Instrument Table
- AFA-TUBE TPX Rack from the respective Instrument Table
- 8 microTUBE Strip Foil Seal (PN 520108)
- 8 AFA-TUBE TPX Strip Caps (PN 500639)
- 1x Tris-EDTA, pH 8.0 (TE buffer, optional for dilution of samples)

Storage

- 1 year at 2 to 8 °C.

Workflow

- It is recommended to dilute the Test Sample (Red Cap) 1:3 in TE buffer. Each Test Sample vial contains 1100 µL of sample. Transfer 1000 µL of the Test Sample to an appropriately sized vial (holds >3.0 mL) containing 2000 µL TE buffer. Mix well. (Note: This can be done for both vials)
 - If the Test Sample is diluted, also 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.
- Fill 50 µL of either the undiluted Test Sample solution (Red Cap) or the 1:3 diluted Test Sample into:
 - **AFA-TUBE TPX Plate:** one plate (PN 520291) placed in the Rack 96 AFA-TUBE TPX Plate (LE220-plus rack PN 500684 or R230 Rack PN 500668).

- We recommend running at least 2 columns of samples for each verification run.
- Process these samples following instrument settings given in **Table 1** for LE220-plus and **Table 2** for R230.
- AFA-TUBE TPX Strips: Three strips (PN 520292) placed in positions 1, 2, and 3 in the Rack 8 AFA-TUBE TPX Strip (LE220-plus rack PN 500685).
- Process these samples following instrument settings given in **Table 1**.

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 your LE220-plus or R230 Focused-ultrasonicator with specific processing Racks and the 96 AFA-TUBE TPX Plate. Ensure you are using the matching rack for your instrument.

Instrument	AFA-TUBE	Rack	Plate Definition	Temp	Sample Volume	PIP	Duty Factor	Cycles per Burst	Dithering	Time
LE220-plus	520291	500684	"LE220plus_500684 96 AFA-TUBE TPX Plate +1.8 offset.plt"	10 °C	50 µL	200 W	25 %	50	1 mm y-dither at 20 mm/s	270 s
LE220-plus	520292	500685	"LE220plus_500685 8 AFA-TUBE TPX Strip +1.8 offset."	10 °C	50 µL	220 W	25 %	50	1 mm y-dither at 20 mm/s	300 s

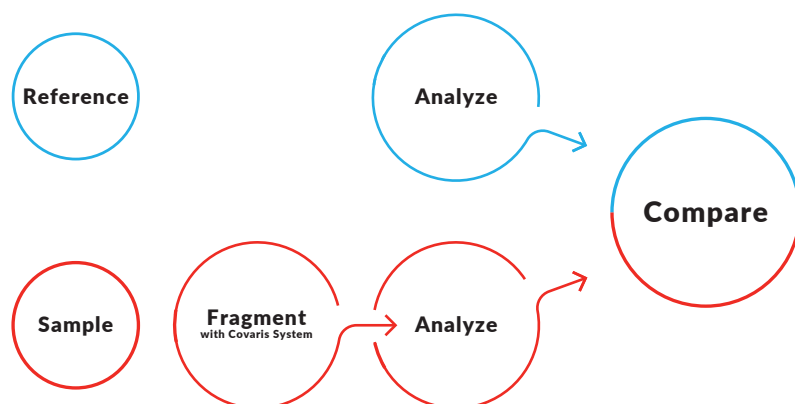
Table 1. Covaris Instrument DNA Shearing Settings for LE220-plus (see **Appendix C**).

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	50 µL	280 W	25 %	50	3 mm y-dither at 20 mm/s	10 s	10 s	14

Table 2. Covaris Instrument DNA Shearing Settings for R230 (see **Appendix D**).

Analysis

- Analyze the fragment size distribution of both Reference and Processed Test Samples on the same chip. Note, two Bioanalyzer chips will be required per verification test. Load the Reference Sample in at least the first and last position used of each chip and the Processed Test Samples in the remaining positions between the Reference Samples.
- Compare fragment size distributions to verify that your Covaris Focused-ultrasonicator is performing correctly.



NOTE: If running undiluted samples on a High Sensitivity assay, dilute all samples 1:3 .before loading on the chip.

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 peak size of the fragment distribution. For the Reference Sample replicates, calculate the average and the Coefficient of Variation. For the sixteen Processed Test Samples, calculate the average and the Coefficient of Variation. Compare the peak size and fragment distribution of the Reference and Processed Test Samples using **Table 3**.

	Average of Processed Test Samples within +/- 15% of Reference Sample	Average of Processed Test Samples more than 15% different from Reference Sample
Coefficient of Variation of Processed Samples < 15%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 15%	Contact Covaris	Contact Covaris
Reference Sample in the 100-300 bp range	Covaris system OK	Contact Covaris
Reference Sample out of the 100-300 bp range	Problem with fragment size distribution analysis	Contact Covaris

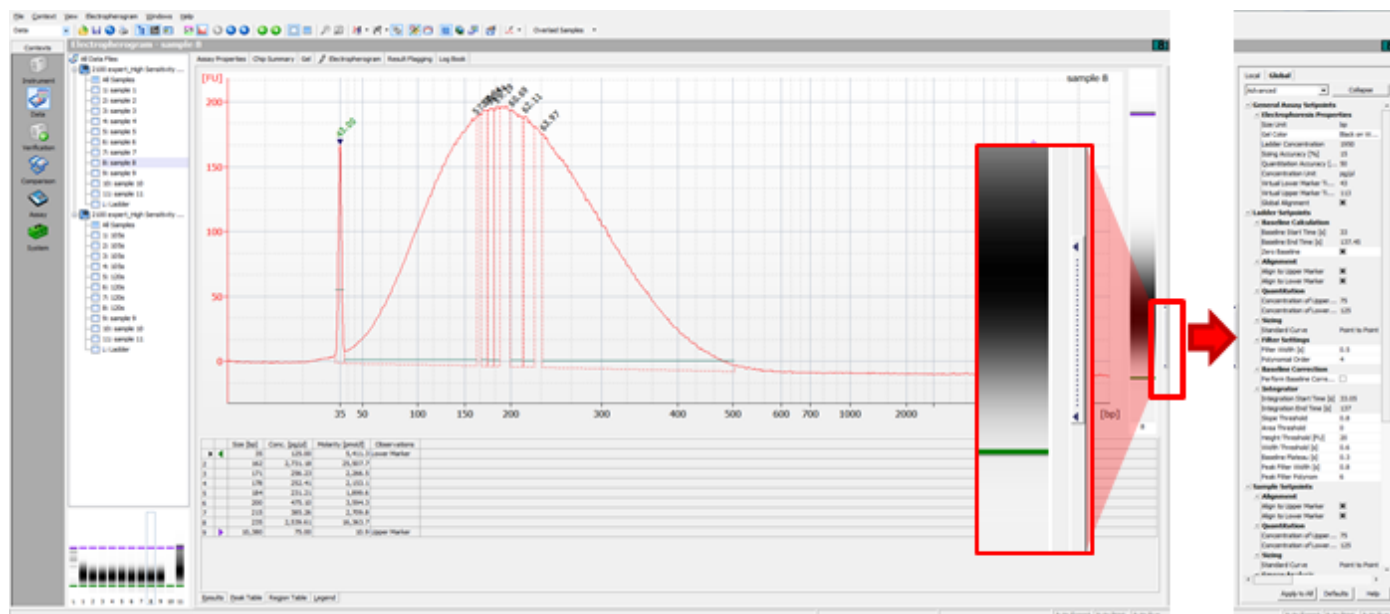
Table 3. Covaris Performance Verification Kit interpretation

Covaris Contact: applicationsupport@covaris.com

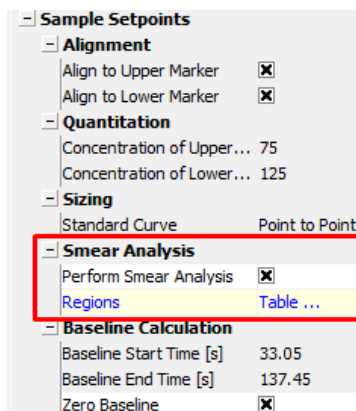
Appendix A: Detailed Instructions for using the Agilent Bioanalyzer 2100

To perform average fragment size (smear) analysis using the Agilent Bioanalyzer 2100, follow the steps provided below:

1. Select the “Global” tab on the right side of the screen and click “Advanced” on the drop-down menu.
 - a. If you cannot see the “Global” tab click on the “.....” to the right side of the screen.

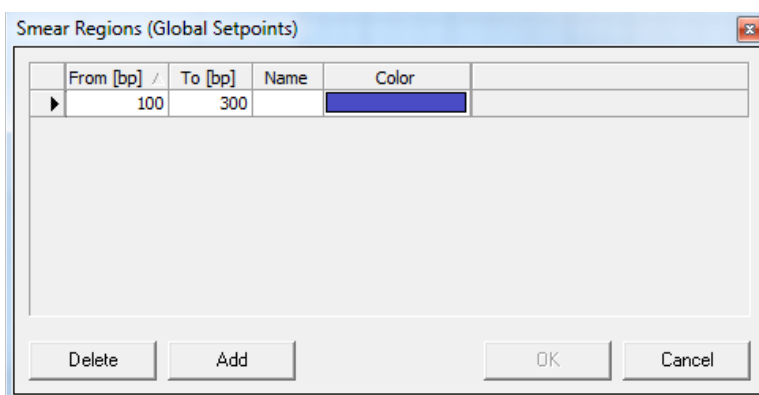


2. Scroll down to “Smear Analysis” under “Sample Setpoints”.



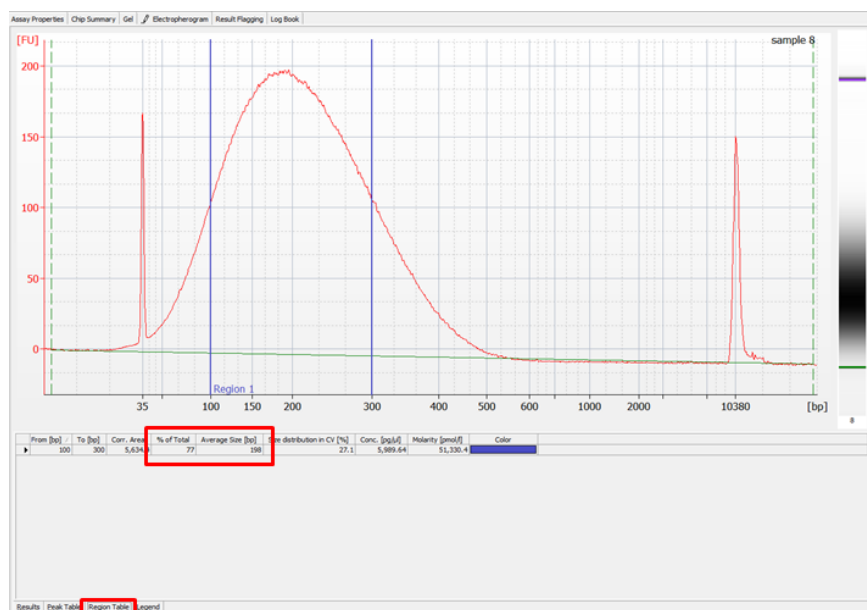
3. Click the box next to “Perform Smear Analysis”.

4. Double click “Table ...” located to the right of “Regions” to open the “Smear Regions” window.



5. Click “Add” to create a new smear region or edit the Smear Region if there is one populated.

6. Double click the values under “From [bp]” and “To [bp]” and enter “100” to “300” then click “OK”.



7. In the main window for each sample, the “Region Table” tab will be populated, and the Region will be marked in the electropherogram.

Note the “% of Total” and “Average Size [bp]” values in the “Region Table”. The “% of Total” for the Reference Standard should be >50%.

CAUTION: A spike in the fragment distribution or a bump in the baseline may occur in some Agilent Bioanalyzer runs. If this occurs, the accuracy of “% of Total” value will be compromised. In this case, please re-run samples on a new chip.

8. Repeat the smear analysis for the Reference Sample and each processed Test Sample.

Appendix B: Troubleshooting

- “% of Total” for the Reference Sample should be > 50%. If it is < 50%, there is a problem with the fragment size distribution analysis. Please check that the Bioanalyzer is functioning correctly then repeat with a new chip.
- If the Coefficient of Variation of the sixteen Processed Test Samples is > 15% or if the average fragment size is > 15% different from the Reference Sample, contact Covaris at applicationsupport@covaris.com
- The “% of Total” takes into account the area below the upper and lower marker, so the results are dependent on sample concentration and do not reflect the actual area of the fragment distribution in the range of interest. It is therefore critical to load the same volume, and the same concentration of Reference and Processed Test Samples.

Appendix C: Screenshot of LE220-plus DNA Shearing Verification Test Method for AFA-TUBE TPX Strips

The screenshot displays the Covaris LE220-plus software interface. At the top, there is a navigation bar with 'HOME', 'HISTORY', 'ABOUT', and 'ADMIN' tabs, and a 'Log Out' link. Below the navigation bar, the current method is 'DNA Shearing Verification AFA TPX Strips_220W_T'. The sample name is 'LE220plus500685 8 AFA-TUBE TPX Strip +1.8 offset'. The power is set to 0.0 W and the temperature to 20.5 °C. The 'Run' button is visible. The 'Process' section shows 'Dithering' and 'Run Time 15m:0s'. The 'Repeat' section shows 'Iterations' set to 30. The 'Treatment' section includes 'Duration (s)' at 10.0, 'Peak Power' at 220.0, 'Duty % Factor' at 25, 'Cycles/Burst' at 50, and 'Avg Power' at 55.0. The 'Rows to Process' section shows a grid of 12 rows (labeled H, G, F, E, D, C, B, A) with columns 1 through 12. The first three rows are highlighted in yellow. The 'System Status' section shows 'Motion Control' and 'Water Works' controls. The 'Creation Notes' section is also visible. At the bottom, the 'Run Time: 15m:0s', 'Temp Set Point: 10°', and 'API Server: 127.0.0.1 : 8100' are displayed.

Appendix D: Screenshot of R230 DNA Shearing Verification Test Method

Example using 2 columns to test:

Revision History

Document PN	Revision	Date	Description of change
010474	E	3/2020	Addition of R230; Updated template
010474	F	5/2020	Addition of 8 AFA-TUBE TPX Strip on the LE220-plus
010474	G	88/2021	Updated R230 Shearing time and added repeats