



**DNA Shearing Verification Kit for  
AFA-TUBE TPX Consumables on the  
LE220-plus, LE220R-plus, and LE220Rsc**

(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 on the LE220-plus, LE220R-plus, and LE220Rsc. 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 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 1,100 µL of Lambda DNA. SDS information is available at: [http://covaris.com/wp-content/uploads/pn\\_010379.pdf](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 PN 5067-4626 or equivalent)
- Focused-ultrasonicator: LE220-plus ([PN 500569](#)), LE220R-plus ([PN 500578](#)), and LE220Rsc ([PN 500652](#))
- [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 2–8 °C

## Workflow

- We recommend diluting the Test Sample 1:3 with TE Buffer for a DNA concentration of 8–10 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.
- 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 50 µL of the 1:3 diluted Test Sample into:
  - 96 AFA-TUBE TPX Plate: one plate ([PN 520291](#)) placed in the Rack 96 AFA-TUBE TPX Plate (LE220-plus rack [PN 500684](#)). 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.
  - 8 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](#)) and 8 AFA-TUBE TPX Strip ([PN 520292](#)). Please follow the settings carefully for the LE220-plus Focused-ultrasonicator with specific processing Racks and the AFA-TUBE TPX consumable.

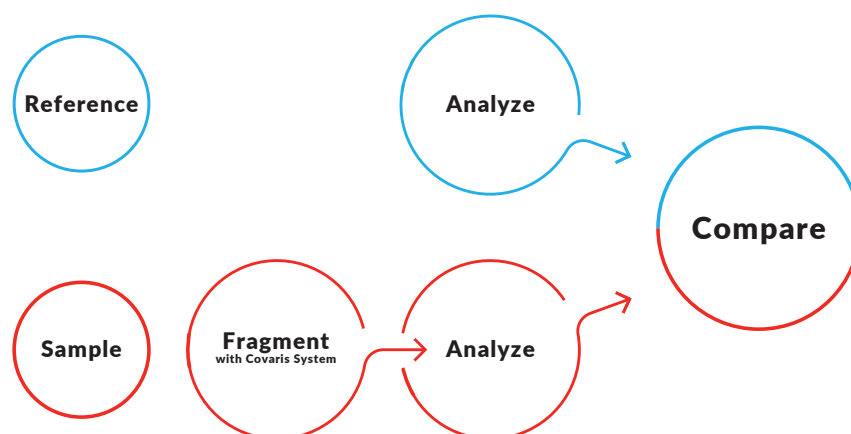
Instrument	AFA-TUBE	Rack	Plate Definition	Temp.	Sample Volume	PIP	Duty Factor	Cycles per Burst	Dithering	Time
LE220-plus	520291	500684	"LE220plus_500684 96 AFA-TUBE TPX Plate +1.8 offset.plt"	10 °C	50 µL	200 W	25%	50	1 mm y-dither at 20 mm/s	270 sec
LE220-plus	520292	500685	"LE220plus_500685 8 AFA-TUBE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

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

### 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 2**.

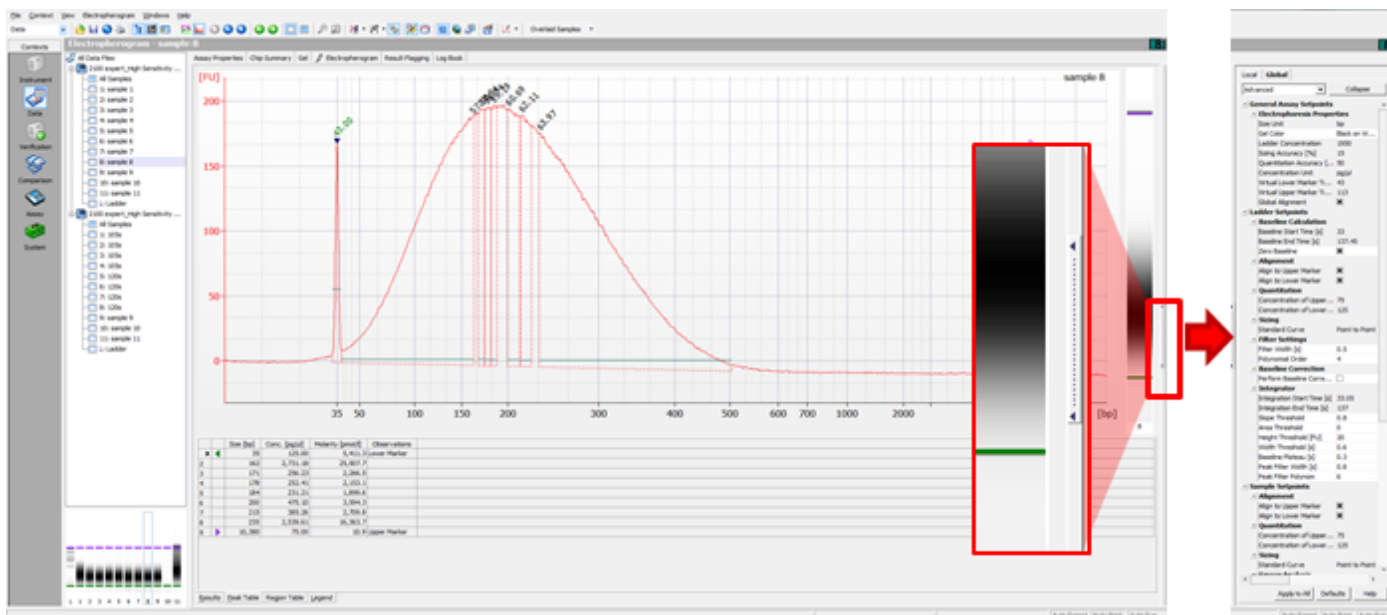
	Average of Processed Test Samples within +/- 15% of Reference Sample	Average of Processed Test Samples more than 15% different from Reference Sample
Coefficient of Variation of Processed Samples < 15%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 15%	Contact Covaris	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 2.** Covaris Performance Verification Kit interpretation. Covaris Contact: [applicationsupport@covaris.com](mailto:applicationsupport@covaris.com).

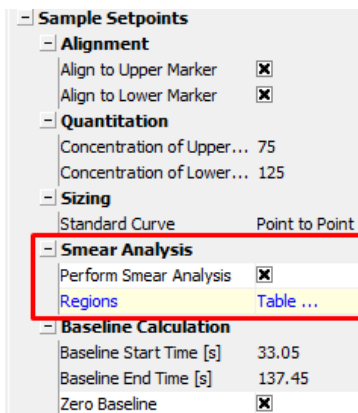
## Appendix A: Detailed Instructions for using the Agilent Bioanalyzer 2100

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.

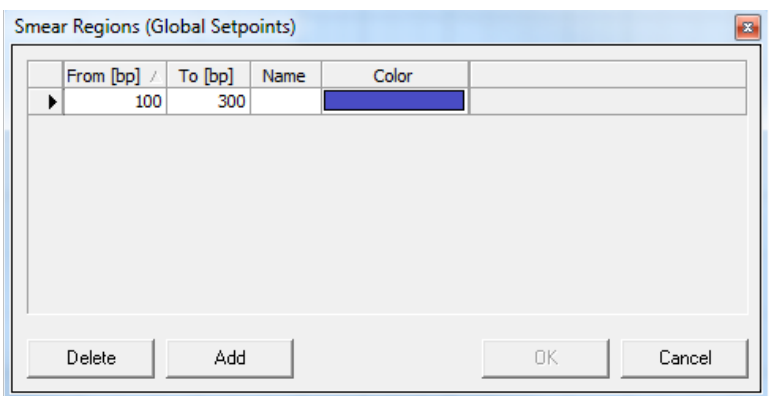


2. Scroll down to “Smear Analysis” under “Sample Setpoints”.



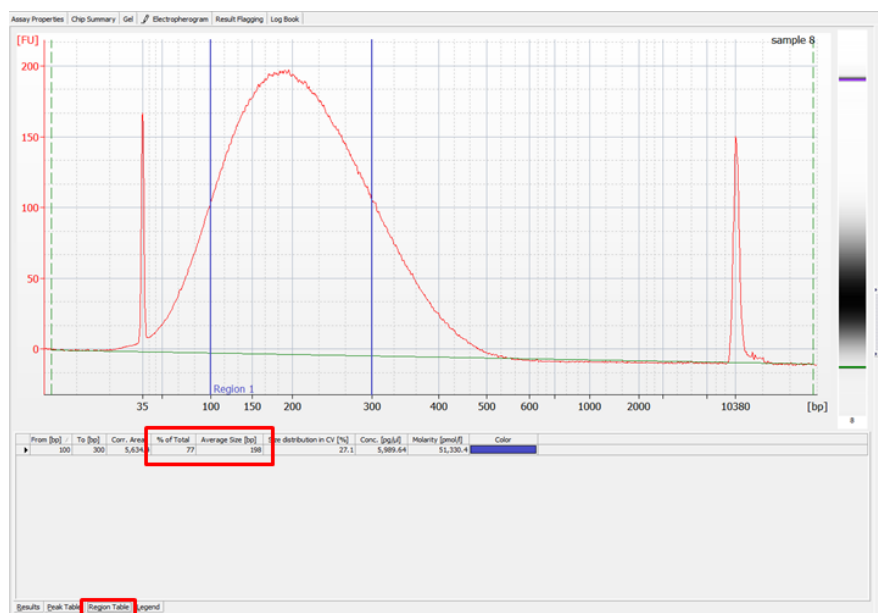
3. Click the box next to “Perform Smear Analysis”.

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”.



- 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.

- 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](mailto: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

