

# Quick Guide

## Proteomics

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This Quick Guide provides cell lysis and protein extraction protocols for the Covaris 8 AFA-TUBE TPX Strip ([PN 520292](#)) and 96 AFA-TUBE TPX Plate ([PN 520291](#)) consumables using three Covaris Focused-ultrasonicator instruments: ML230, LE220-plus, and LE220Rsc. Please contact Covaris at [ApplicationSupport@covaris.com](mailto:ApplicationSupport@covaris.com) for protocols with other Focused-ultrasonicators.

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

- Temperature:  $\pm 3$  °C
- Sample Volume:
  - 8 AFA-TUBE TPX Strip:  $\pm 2.5$   $\mu$ l
  - 96 AFA-TUBE TPX Plate:  $\pm 2.5$   $\mu$ l
- Water Level:  $\pm 1$  mm

### Sample Guidelines

- Input: For both 8 AFA-TUBE TPX Strips and 96 AFA-TUBE TPX Plates
- Formalin Fixed Paraffin Embedded (FFPE) Scrolls - up to 10  $\mu$ m x ~ 500 mm<sup>2</sup>
- < 5 mg of fresh frozen tissues
- Mammalian cells (1,000 to 100,000)
- Buffers: Tissue Lysis Buffer (TLB) (PN 520284)
- **WARNING:** DO NOT use the AFA-TUBE TPX Strips or Plates for long term sample storage. Samples should be transferred after processing.

### Instrument Setup

- Refer to the specific instrument manual for complete setup.
- AFA-TUBE TPX Strips and Plates have specific racks associated with them for each instrument.

### Instrument Settings

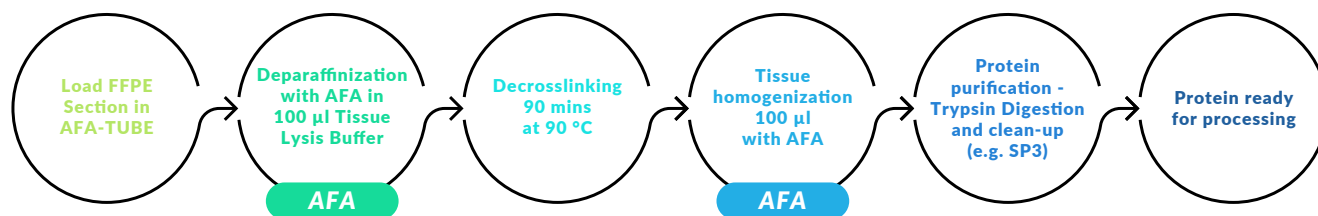
- Recommended settings are subject to change without notice.

For updates to this document, please click [here](https://www.covaris.com/wp/wp-content/uploads/pn_010542.pdf) ([https://www.covaris.com/wp/wp-content/uploads/pn\\_010542.pdf](https://www.covaris.com/wp/wp-content/uploads/pn_010542.pdf)).

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## Workflow for FFPE Scrolls



## Protocol for FFPE Scrolls

Instructions for processing FFPE Scrolls using Tissue Lysis Buffer.

- Load up to ~ 10 µm x ~500 mm<sup>2</sup> FFPE Scroll in each of the test wells.
- The recommended volume of TLB is 100 µl but can be changed from 75 to 150 µl based on the size of the scroll. Load 100 µl of Tissue Lysis Buffer in each of the test wells. Ensure that the scrolls are fully submerged in Tissue Lysis Buffer.
- **Deparaffinization:**
  - Process the test wells with the AFA conditions for 'Emulsification' depicted in the AFA settings tables below.
- **Decrosslinking:**
  - Place the AFA-TUBE TPX Plate or Strip into a thermocycler set at 90 °C for 90 minutes.
- **Homogenization:**
  - Process the test wells with the AFA conditions for 'Homogenization' depicted in the AFA settings table in the following pages.

Protein extraction can be analyzed at this point. Centrifuge the AFA-TUBE TPX consumable at 3,000 x g for 5 mins (Plate) or 5000 x g for 5 mins (Strip). Remove the supernatant and use it for quantitative (BCA Assay) and/or qualitative analysis (SDS-PAGE). Please refer to **Appendix A: Protein Extraction Verification** for additional information.

## FFPE Workflow - ML230 Settings

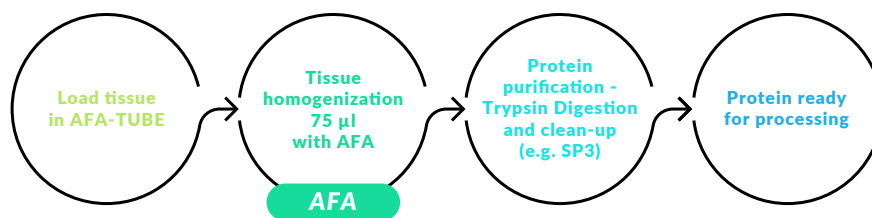
Vessel	8 AFA-TUBE TPX Strip (PN 520292)	
Buffer	TLB (PN 520284)	
Rack	PS ML230 Rack 8 AFA-TUBE TPX Strip (PN 500699)	
Temperature (°C)	20	
SonoLab Version	10.1	
Step	Deparaffinization	Homogenization
Plate Definition (ML230_)	500699 Rack_520292 Strip_17WL_12.7OS	500699 Rack_520292 Strip_17WL_12.7OS
Dithering	3 mm Y-dither @ 20 mm/s	3 mm Y-dither @ 20 mm/s
Volume (µl)	100	
Peak Power (W)	350	350
Duty Factor (%)	25	25
Cycles per Burst	200	200
Time (sec)	300	300

## FFPE Workflow - LE220-plus and LE220Rsc Settings

Vessel	96 AFA-TUBE TPX Plate (520291)	
Buffer	TLB (PN 520284)	
Rack	PS Rack 96 AFA-TUBE TPX Plate (PN 500622)	
Temperature (°C)	20	
SonoLab Version	8.5	
Step	Deparaffinization	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500622 Rack_520291 Plate_53WL_-5.0OS	500622 Rack_520291 Plate_55WL_-2.2OS
Dithering	3 mm Z-dither @ 10 mm/s	1 mm Y-dither @ 20 mm/s
Volume (µl)	100	
Peak Power (W)	350	350
Duty Factor (%)	25	25
Cycles per Burst	200	200
Time (sec)	300	300

Vessel	8 AFA-TUBE TPX Strip (PN 520292)	
Buffer	TLB (PN 520284)	
Rack	PS Rack 96 AFA-TUBE TPX Strip (PN 500698)	
Temperature (°C)	20	
SonoLab Version	8.5	
Step	Deparaffinization	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500698 Rack_520292 Strip_53WL_-5.0OS	500698 Rack_520292 Strip_55WL_-2.2OS
Dithering	3 mm Z-dither @ 10 mm/s	1 mm Y-dither @ 20 mm/s
Volume (µl)	100	
Peak Power (W)	350	350
Duty Factor (%)	25	25
Cycles per Burst	200	200
Time (sec)	300	300

## Workflow for Tissue Lysis



## Protocol for Tissue Lysis

Instructions for processing tissue using Tissue Lysis Buffer.

- Load up to 5 mg of tissue into each of the test wells.
- Load 75 µl of Tissue Lysis Buffer in each of the test wells. Ensure that the tissue is fully submerged in Tissue Lysis Buffer.
- **Homogenization:**
  - Process the test wells with the AFA conditions for 'Homogenization' depicted in the AFA settings table in the following pages.

Protein extraction can be analyzed at this point. Centrifuge the AFA-TUBE TPX consumable at 3,000 x g for 5 mins (Plate) or 5000 x g for 5 mins (Strip). Remove the supernatant and use it for quantitative (BCA Assay) and/or qualitative analysis (SDS-PAGE). Please refer to **Appendix A: Protein Extraction Verification** for additional information.

## Tissue Lysis Workflow - ML230 Settings

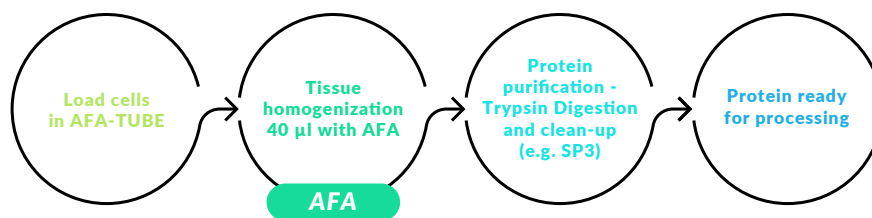
Vessel	8 AFA-TUBE TPX Strip (PN 520292)
Buffer	TLB (PN 520284)
Rack	PS ML230 Rack 8 AFA-TUBE TPX Strip (PN 500699)
Temperature (°C)	20
SonoLab Version	10.1
Step	Homogenization
Plate Definition (ML230_)	500699 Rack_520292 Strip_17WL_12.7OS
Dithering	3 mm Y-dither @ 20 mm/s
Volume (µl)	75
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300

## Tissue Lysis Workflow - LE220-plus and LE220Rsc Settings

Vessel	96 AFA-TUBE TPX Plate (520291)
Buffer	TLB (PN 520284)
Rack	PS Rack 96 AFA-TUBE TPX Plate (PN 500622)
Temperature (°C)	20
SonoLab Version	8.5
Step	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500622 Rack_520291 Plate_53.5WL_-2.2OS
Dithering	1 mm Y-dither @ 20 mm/s
Volume (µl)	75
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300

Vessel	8 AFA-TUBE TPX Strip (PN 520292)
Buffer	TLB (PN 520284)
Rack	PS Rack 96 AFA-TUBE TPX Strip (PN 500698)
Temperature (°C)	20
SonoLab Version	8.5
Step	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500698 Rack_520292 Strip_53.5WL_-2.2OS
Dithering	1 mm Y-dither @ 20 mm/s
Volume (µl)	75
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300

## Workflow for Cell Lysis



## Protocol for Cell Lysis

Instructions for processing cells using Tissue Lysis Buffer .

- Load up to  $10^5$  cells into each of the test wells.
- Load 40 µl of Tissue Lysis Buffer in each of the test wells.
- **Homogenization:**
  - Process the test wells with the AFA conditions for 'Homogenization' depicted in the AFA settings table in the following pages.

Protein extraction can be analyzed at this point. Centrifuge the AFA-TUBE TPX consumable at 3,000 x g for 5 mins (Plate) or 5000 x g for 5 mins (Strip). Remove the supernatant and use it for quantitative (BCA Assay) and/or qualitative analysis (SDS-PAGE). Please refer to **Appendix A: Protein Extraction Verification** for additional information.

## Cell Lysis Workflow - ML230 Settings

Vessel	8 AFA-TUBE TPX Strip (PN 520292)
Buffer	TLB (PN 520284)
Rack	PS ML230 Rack 8 AFA-TUBE TPX Strip (PN 500699)
Temperature (°C)	20
SonoLab Version	10.1
Step	Homogenization
Plate Definition (ML230_)	500699 Rack_520292 Strip_17WL_12.7OS
Dithering	3 mm Y-dither @ 20 mm/s
Volume (µl)	40
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300

## Cell Lysis Workflow - LE220-plus and LE220Rsc Settings

Vessel	96 AFA-TUBE TPX Plate (520291)
Buffer	TLB (PN 520284)
Rack	PS Rack 96 AFA-TUBE TPX Plate (PN 500622)
Temperature (°C)	20
SonoLab Version	8.5
Step	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500622 Rack_520291 Plate_53.5WL_-2.2OS
Dithering	1 mm Y-dither @ 20 mm/s
Volume (µl)	40
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300

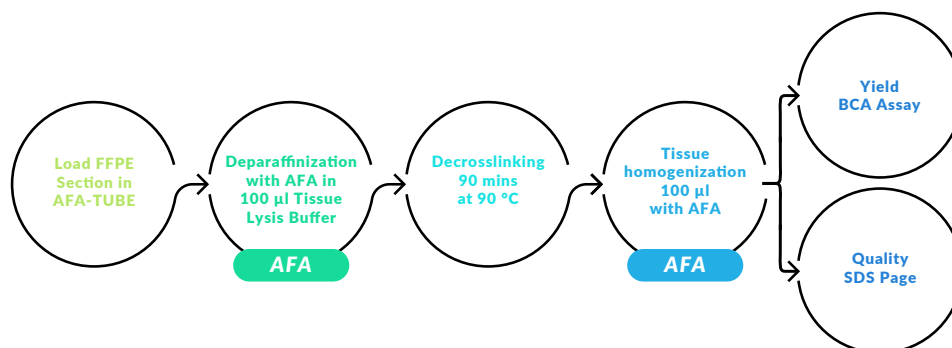
Vessel	8 AFA-TUBE TPX Strip (PN 520292)
Buffer	TLB (PN 520284)
Rack	PS Rack 96 AFA-TUBE TPX Strip (PN 500698)
Temperature (°C)	20
SonoLab Version	8.5
Step	Homogenization
Plate Definition (LE220plus_ or LE220PRSC_)	500698 Rack_520292 Strip_53.5WL_-2.2OS
Dithering	1 mm Y-dither @ 20 mm/s
Volume (µl)	40
Peak Power (W)	350
Duty Factor (%)	25
Cycles per Burst	200
Time (sec)	300



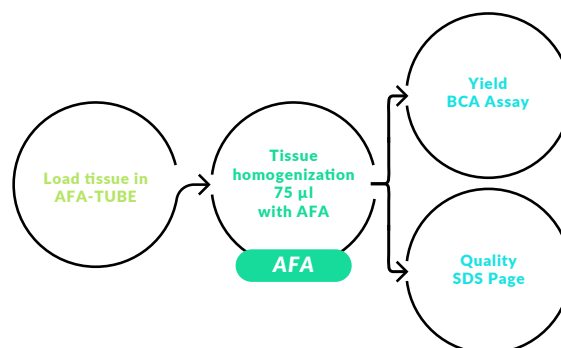
## Appendix A: Protein Extraction Verification

The process verification can be performed following these protocols.

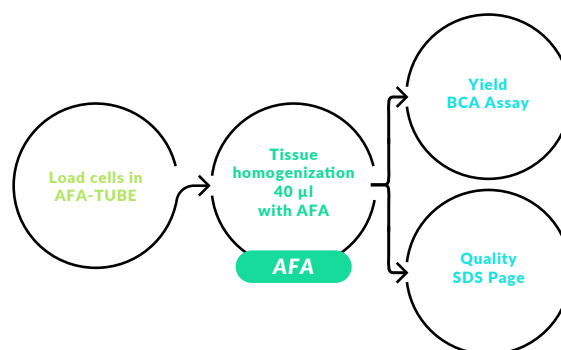
**FFPE Workflow Verification:** Covaris can provide FFPE scrolls to be used as internal standards.



### Tissue Workflow Verification



### Cell Lysis Workflow Verification



The quantitative performance of the protein extraction workflow with Covaris buffer can be tested using BCA Assay (Pierce™ BCA Protein Assay Kit, PN 23227 from ThermoFisher Scientific). It is recommended that the solution be diluted 1:5 with deionized water before performing the BCA Assay. In addition, different dilutions of the BCA Assay Standard should be performed using the same diluent (TLB buffer diluted 1:5 using deionized water). The qualitative performance can be tested using SDS-PAGE (4-20% Criterion TGX Stain-Free Protein Gel, PN 5678093 from Bio-Rad). Quantitative and qualitative data for control samples are shared upon request. Please reach out to [ApplicationSupport@covaris.com](mailto:ApplicationSupport@covaris.com) with any questions.

## Technical Assistance

- By telephone (+1 781.932.3959) during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at [ApplicationSupport@covaris.com](mailto:ApplicationSupport@covaris.com)

## Revision History

Part Number	Revision	Date	Description of Change
010542	A	12/2020	Initial release