Product Insert: 96 microTUBE Plates

Introduction
The 96 microTUBE and 96 microTUBE-50 Plates are a ready-to-use sample processing consumable optimized for use with Covaris Adaptive Focused Acoustics® (AFA®). Each plate contains 96 microTUBEs and allows great flexibility when processing samples in varying batch sizes. Each tube contains an AFA fiber that improves reproducibility, promotes an isothermal process, reduces fragmenting times, and allows small sample volumes to be processed.

The plates are manufactured with a pre-slit adhesive foil for dispensing samples. There are 2 versions of the 96 microTUBE and 96 microTUBE-50 Plates that include an updated Thin Foil seal. This is the only difference between the plates. An additional foil seal is included with each plate and the Thin Foil plates have an additional Thin Foil seal. It is recommended to use the Thin Foil for automation. The plates have a barcode for traceability.

E220 and LE-series
The 96 microTUBE and 96 microTUBE-50 Plates are compatible with the E220 and L-Series Covaris instruments and do not require the use of an instrument specific rack. For the LE-series instruments, Rack-XT 96 microTUBE Plate is recommended for chromatin shearing applications. Refer to the Covaris-certified Consumable Guide for further information (https://covaris.com/wp-content/uploads/M020065.pdf).

R230
The 96 microTUBE Plate is compatible with the R230. It requires an instrument specific polysulfone (PSU) Rack (PN 500750). It is not compatible with chromatin shearing applications and is only validated for use in NGS sample processing workflows.

The 96 microTUBE Plates comply with the ANSI/SBS-4 standard for 96 well microplates. They are designed for use with automated 1, 8, and 96 channel pipettes.

DNA shearing protocols with the 96 microTUBE Plates are also instrument specific. Do not store sheared samples in the microTUBEs. Recommended instructions are subject to change without notice.

Ordering Information
- 96 microTUBE-50 AFA Fiber Plate (1) (PN 520168)
- 96 microTUBE-50 AFA Fiber Plate (10) (PN 520169)
- 96 microTUBE-50 AFA Fiber Thin Foil Plate (1) (PN 520232)
- 96 microTUBE-50 AFA Fiber Thin Foil Plate (10) (PN 520233)
- 96 microTUBE Plate (1) (PN 520078)
- PSU Rack R230 TPX Plate & 130 Plate (PN 500750)
- 96 microTUBE Plate (10) (PN 520069)
- 96 microTUBE AFA Fiber Thin Foil Plate (1) (PN 520230)
- 96 microTUBE AFA Fiber Thin Foil Plate (10) (PN 520231)
- Rack-XT 96 microTUBE Plate (PN 520239) (for chromatin shearing on the LE-series instruments)
- 96 microTUBE Plate Foil Seal (25) (PN 520073)
- 96 microTUBE Plate Thin Foil Seals (25) (PN 520235)

DNA Shearing Protocols
Operating Limits and Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Temperature (water bath)</td>
<td>4 °C minimum; 25 °C maximum</td>
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</tbody>
</table>
| Recommended Sample Volume         | • 96 microTUBE-50: 55 µL ± 2.5 µL  
• 96 microTUBE: 130 µL ± 5 µL 
| Centrifuge                        | 180 RCF                        |
| Storage                           | Room temperature (15 ºC to 30 ºC) |

**CAUTION:** All Covaris microTUBEs must operate within energy constraints. The power maximum levels are guides and should not be exceeded. Operating outside of these limits or limits published in Covaris protocols may compromise the integrity of the microTUBE.

**E220**

- **Peak Incident Power**
  - 96 microTUBE-50: 100 W maximum
  - 96 microTUBE: 175 W maximum
- **Duty Factor**
  - 96 microTUBE-50: 30% maximum
  - 96 microTUBE: 10% maximum
- **Intensifier**
  - 500141 required, installed on transducer (See Appendix A for details)
- **Water level (RUN scale)**
  - 96 microTUBE-50: Level 0
  - 96 microTUBE: Level 6
- **E220 Plate Definitions**
  - 96 microTUBE-50: E220_520168 96 microTUBE-50 Plate -10.5mm offset
  - E220_520232 96 microTUBE-50 Thin Foil Plate -10.5mm offset
  - 96 microTUBE: E220_520078 96 microTUBE Plate -6mm offset
  - E220_520230 96 microTUBE Plate -6mm offset

**LE-Series**

- **Peak Incident Power (PIP)**
  - 450 W maximum
- **Duty Factor**
  - 30% maximum
- **LE220 Water level (RUN scale)**
  - 96 microTUBE-50: Level -2
  - 96 microTUBE: Level 6
- **LE220 Plate Definition**
  - 96 microTUBE-50: LE220_520168 96 microTUBE-50 Plate -12mm offset
  - 96 microTUBE-50 Thin Foil: LE220_520232 96 microTUBE-50 Thin Foil Plate -12mm offset
  - 96 microTUBE: LE220_520078 96 microTUBE Plate -4mm offset
  - 96 microTUBE Thin Foil: LE220_520230 96 microTUBE Plate -4mm offset
- **LE220-plus/LE220R-plus Plate Definition**
  - 96 microTUBE-50: LE220plus_520168 96 microTUBE-50 Plate -12mm offset
  - 96 microTUBE-50 Thin Foil: LE220plus_520232 96 microTUBE-50 Thin Foil Plate -12mm offset
  - 96 microTUBE: LE220plus_520078 96 microTUBE Plate -4mm offset
  - 96 microTUBE Thin Foil: LE220plus_520230 96 microTUBE Plate -4mm offset
- **LE220Rsc**
  - 96 microTUBE-50: LE220PRSC_520168 96 microTUBE-50 Plate -12mm offset
  - 96 microTUBE-50 Thin Foil: LE220PRSC_520232 96 microTUBE-50 Thin Foil Plate -12mm offset
  - 96 microTUBE: LE220PRSC_520078 96 microTUBE Plate -4mm offset
  - 96 microTUBE Thin Foil: LE220PRSC_520230 96 microTUBE Plate -4mm offset

**NOTE:** If the plate definition is not present on the system, contact Covaris Technical Support (TechSupport@covaris.com) with the system serial number.
Nominal Rack Dimensions (E220 and LE-series)

- Overall Plate Height (top of tubes) 19.0 mm above mounting plane
- Tube center-to-center spacing 9.0 mm (SBS standard)
- Interior clearance diameter 3.0 mm (maximum tip diameter 15 mm from end)
- 96 microTUBE: Tube depth 15.0 mm (bottom is 4.0 mm above mounting plane) (pictured)
- 96 microTUBE-50: Tube depth 7.0 mm (bottom is 4.0 mm above mounting plane)

Recommended Pipette Tips

To avoid binding against the tube interior when fully inserted into the microTUBE, use pipette tips that maintain a diameter no greater than 3 mm within 15 mm of their dispensing end.

**NOTE:** Many robotic systems use proprietary tips so this diameter should be verified prior to use.

**CAUTION:** In automated liquid handling systems, friction between the 96 pipette tips and foil seal may cause the rack to lift off the deck as pipette tips are raised. A hold-down clamp for SBS plates is recommended.

Please refer to the following documents for pipette recommendations.

Instructions for Use

1. Remove the shrink film and desiccant pack prior to use.

2. Do not remove the pre-slit foil seal from the plate. The foil seals can be pierced with pipette tips before sample addition if desired.

   **NOTE:** To prevent the AFA Fiber from falling out, do not tip the plate once the seal has been pierced.

3. Aspirate the recommended volume of sample and add it to the microTUBE. A single-channel or multi-channel pipette may be used. Ensure the foil is pierced so there is ample air exchange when dispensing the sample.

4. Seal the plate with the enclosed foil seal. Only microTUBEs that contain sample need to be sealed. The foil seal may be cut to the appropriate size if less than 96 microTUBEs are used.

5. Process the samples according to the appropriate protocol for the respective instrument.

6. To retrieve the sample, the top foil seal can be pierced with pipette tips.

7. If fewer than 96 samples are processed, save the plate and remaining foil seal and store at 15 to 30 °C.

Recommended Sequence for Automated Use

1. **Pierce the foil seal:** Load pipette tips and press the tips through the foil seal, fully piercing the foil by going to the bottom of the tube. This will allow air to flow out of the tube during filling. This helps to avoid bubble formation.

   **NOTE:** To prevent the AFA Fiber from falling out, do not tip the plate once the seal has been pierced.

2. **Fill the tubes:** Aspirate sample and dispense into the pre-pierced, open microTUBEs. Since the recommended sample and tube volume are nearly identical, be careful to not overflow the microTUBE by inserting the tip too far down into the tube during dispensing. To avoid fluid displacement and bubble formation, either 1.) extract the tip as the sample is dispensed, or 2.) dispense slowly with the tip located just below the top of the tube.

3. **Reseal the plate for processing:** Remove the backing from the spare foil seal and carefully align it over the plate. Using a sealing paddle, roller, or your fingers, thoroughly press the new seal over the pierced seal, verifying that the seal is adhered to the top of each tube. The plate is now ready to be processed.

4. **Sample aspiration:** After processing, the samples are ready to be aspirated. Samples should be aspirated as soon as is practical after treatment. Do not use the 96 microTUBE Plate for long term storage. Be careful to not overflow the microTUBE by inserting the tip too far down into the tube. Air must also be allowed to enter the tube during sample withdrawal. Carefully pierce the foil and aspirate as you lower the tip into the tube, maintaining contact with the fluid to avoid aspirating air. You may have to raise the tip once or twice during aspiration to allow the tube to vent.

5. **Centrifugation:** If necessary, centrifugation is permitted (up to 180 g (RCF)). This is about 1000 RPM in a benchtop centrifuge with a swinging bucket rotor. Do not stack plates in the centrifuge.

Revision History

<table>
<thead>
<tr>
<th>Document Part #</th>
<th>Revision</th>
<th>Date</th>
<th>Description of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>010112</td>
<td>N</td>
<td>8/2017</td>
<td>Add the names and the plate definitions for S20230 &amp; S20232</td>
</tr>
<tr>
<td>010112</td>
<td>O</td>
<td>6/2020</td>
<td>Power limits for the 96 microTUBE-50 Plate part numbers; Add Introduction; Update document template.</td>
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<tr>
<td>010112</td>
<td>P</td>
<td>10/2020</td>
<td>Update hyperlinks</td>
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<tr>
<td>010112</td>
<td>Q</td>
<td>3/2022</td>
<td>Add R230 and 96 microTUBE plate</td>
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</table>
Appendix A: Removing or Installing the Intensifier (Covaris PN 500141) from a Covaris E System

The 500141 Intensifier is a small inverted stainless steel cone centered over the E-Series transducer by four stainless wires. The wires are held in place by a black plastic ring pressed into the transducer well.

If an AFA protocol requires “no Intensifier”, please remove the Intensifier, using the following steps:

1. Empty the water bath. Start the instrument and start the SonoLab™ software.
2. Wait for the homing sequence to complete (the transducer will be lowered with the rack holder at the home position, allowing easy access to the Intensifier).
3. Grasp opposite sides of plastic ring and gently pull the entire assembly out of the transducer well. Do not pull on the steel cone or the wires. The ring is a friction fit in the well – no hardware is used to hold it in place.

If a protocol requires the Intensifier to be present, simply reverse this process:

4. Align the black plastic ring with the perimeter of the transducer well. Note that the flat side of the center cone (marked UP) should be facing up (away from the transducer).
5. Gently press each section of the ring into the well until the ring is seated uniformly in contact with the transducer, with approximately 2 mm of the ring evenly exposed above the transducer assembly. Do not press on the cone or wires. The rotation of the ring relative to the transducer assembly is not important.
6. Refill the tank. Degas and chill the water before proceeding.

Technical Support – Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone
  - United States: Tel: +1 781.932.3959 during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Eastern Standard Time (EST), Greenwich Mean Time (GMT-05:00)
  - Europe: Tel: 44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m., Monday through Friday, Greenwich Mean Time
- E-mail queries to techsupport@covaris.com or applicationsupport@covaris.com