Covaris®

a PerkinElmer Company

# truCOVER® Total RNA Library Prep Kit

## (PN 520372KIT & 520373KIT)

## Table of Contents

## Revision History

| Part Number | Revision | Date | Description of Change |
|---|---|---|---|
| 010716 | A | 12/2025 | Initial Release |
| 010716 | B | 2/2026 | Clarification of Wording |
| 010716 | C | 2/2026 | Part Number Correction |

## Intended Use

The ***truCOVER Total RNA Library Prep Kit*** is intended to generate RNA libraries compatible with next generation sequencing platforms.

## Product Description

The truCOVER Total RNA Library Prep Kit is designed for generating RNA libraries from total RNA isolated from FFPE tissues, regardless of quality. FFPE RNA is often highly fragmented with size distributions varying widely between samples. As such, these samples require optimized processes for initial RNA fragmentation. If using chemical hydrolysis for this step, RNA is heated in buffers containing divalent cations. Chemical RNA hydrolysis methods are dependent on temperature and incubation time and therefore optimal fragmentation is difficult to achieve using a chemical hydrolytic approach with RNA isolated from FFPE samples.

Mechanical fragmentation using Adaptive Focused Acoustics® (AFA®), is a more robust process and overcomes the workflow variations associated with chemical hydrolysis. The AFA settings for the truCOVER RNA Library workflow in this kit shear RNA to a recommended 350 nucleotide fragments. FFPE RNA that is already degraded to smaller lengths will not be impacted by AFA, unlike with chemical fragmentation. It is recommended to use FFPE RNA with $DV_{200}$ scores of greater than 30% to take advantage of this feature and achieve libraries with >200 bp cDNA insert sizes.

The truCOVER RNA Library Prep Kit incorporates a high-performance ribosomal RNA-depletion step optimized for degraded RNA that seamlessly connects to the reverse transcription and cDNA synthesis step, preserving strand-specificity. Adaptor indices are incorporated by amplification after an interim magnetic bead cleanup step. After a final cleanup step, the library is ready to undergo quality control (QC) procedures and be pooled with other samples for sequencing. The schematic workflow is shown in ***Figure 1***.

Recommended total FFPE RNA input range is 50–500 ng. Inputs as low as 10 ng can be used but resulting library quality and yield is strongly dependent on the degree of initial RNA quality.

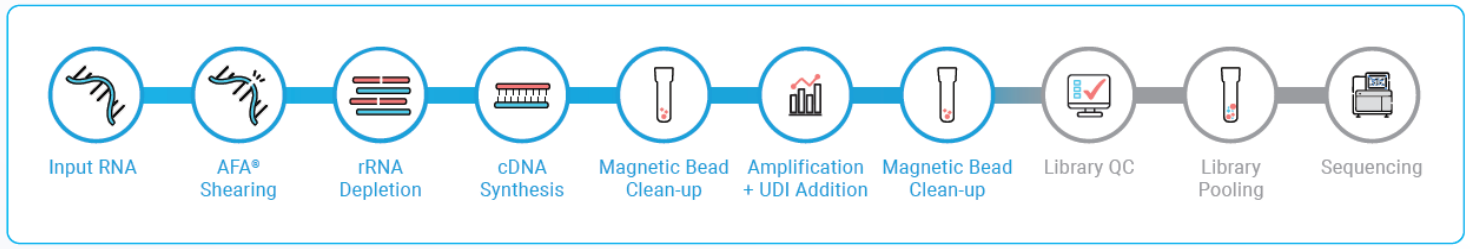


**Figure 1.** truCOVER RNA Library Prep Workflow.

## Important Considerations

Isolated FFPE RNA should be dissolved in buffers free of additives such as detergents, salts and preservatives. Supported elution buffers are nuclease-free water, 10 mM Tris (pH 7) with 0.1 mM EDTA (low-TE), or 5-10 mM Tris (pH 7.0).

## Kit and Contents

This kit contains reagents for processing 24 (520373KIT) or 96 (520372KIT) samples. The truCOVER RNA Library Prep Kit is delivered in 2 boxes.

- **Box 1** of 2 contains Library Prep reagents that must be stored at -20°C
- **Box 2** of 2 contains Magnetic Beads for Library Clean-up and must be stored at 4–8°C

| Kit Components Box 1 of 2 | Quantity (250373KIT) | Quantity (250372KIT) |
|---|---|---|
| Nuclease Free Water | 1.5 mL | 1.5 mL (2x) |
| rRNA Depletion Mix | 12 µL (x3) | 120 µL |
| Hexamer RT Primer | 60 µL | 240 µL |
| Oligo (dT) Primer | 30 µL | 120 µL |
| 10X RT Buffer | 150 µL | 580 µL |
| 10X $MgCl_2$ | 150 µL | 580 µL |
| DTT | 20 µL | 80 µL |
| dNTP Mix | 55 µL | 235 µL |
| Reverse Transcriptase | 36 µL (2x) | 150 µL (2x) |
| RNase Inhibitor | 20 µL | 96 µL |
| Sample ID Primer (Plate) | 24 wells | 96 wells |
| 8-cap Strips | 3 | 12 |
| Amplification Mix | 720 µL | 2.8 mL |

| Kit Components Box 2 of 2 | Quantity (250373KIT) | Quantity (250372KIT) |
|---|---|---|
| Bead Purification Reagent | 3.5 mL | 14 mL |
| Elution Buffer | 2.5 mL | 10 mL |

A Covaris RNA UDI Index plate is required to complete the truCOVER® Total RNA Library Prep workflow and is sold separately. Covaris offers 8 plates, each with 96 different indices to facilitate high throughput multiplexing.

| UDI Index Plate | Part Number |
|---|---|
| Covaris RNA UDI Index Set A (96) | 520378 |
| Covaris RNA UDI Index Set B (96) | 520379 |
| Covaris RNA UDI Index Set C (96) | 520380 |
| Covaris RNA UDI Index Set D (96) | 520381 |
| Covaris RNA UDI Index Set E (96) | 520382 |
| Covaris RNA UDI Index Set F (96) | 520383 |
| Covaris RNA UDI Index Set G (96) | 520384 |
| Covaris RNA UDI Index Set H (96) | 520385 |

## Instrumentation, Accessories, and Consumables

| Product Name | Part Number | Instrument, Accessory, or Consumable |
|---|---|---|
| R230 Focused-ultrasonicator | 500620 | Instrument |
| R230 Rack AFA-TUBE TPX Plate | 500668 | Accessory |
| PSU Rack R230 8 AFA-TUBE TPX Strip | 500723 | Accessory |
| ML230 Focused-ultrasonicator | 500656 | Instrument |
| ML230 Rack 8 AFA-TUBE TPX Strip | 500660 | Accessory |
| LE220-plus Focused-ultrasonicator | 500569 | Instrument |
| LE220plus Rack 8 AFA-TUBE TPX Strip | 500685 | Accessory |
| 8 AFA-TUBE TPX Strip | 520292 | Consumable |
| 8 AFA-TUBE TPX Strip Caps | 500639 | Consumable |

## Instrument Setup Before Starting Library Prep

These preparations should be finished before starting the Library Prep section.

**R230, ML230, and LE220-plus Focused Ultrasonicators**

Refer to the appropriate Instrument Manual and User Guide found on the Covaris website for complete setup.

1. Turn on Focused-ultrasonicator prior to starting reagent preparation step.
2. Check for SonoLab software updates and use the latest available version. Consumables may require a SonoLab update to run protocol. Current versions of SonoLab can be found at https://www.covaris.com/.
3. Install appropriate rack (see accessories table above) into the instrument if not already done.
4. Create the "RNA Shearing" method for your Covaris Focused-ultrasonicator in SonoLab (***see table below***). The settings will shear RNA to fragments with an optimal size for RNA sequencing.

| Instrument | R230 | ML230 | LE220-plus |
|---|---|---|---|
| SonoLab | 10.0.2 | 10.1.1 | 10.2.1 |
| Consumable PN | 8 AFA-TUBE TPX Strip (PN 520292) | 8 AFA-TUBE TPX Strip (PN 520292) | 8 AFA-TUBE TPX Strip (PN 520292) |
| Rack PN | Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668) | ML230 Rack 8 AFA-TUBE TPX Strip (PN 500660) | LE220plus Rack 8 AFA-TUBE TPX Strip (PN 500685) |
| Holder PN | PSU Rack R230 8 AFA-TUBE TPX Strip (PN 500723) | N/A | N/A |
| Plate Definition | R230_500723 PSU Holder 8 AFA-TUBE TPX Strip +0.5 offset | ML230_500660 Rack 8 AFA-TUBE TPX Strip +12.7 mm offset | LE220plus-500685 8 AFA-TUBE TPX Strip 1.8 offset |
| Volume | 5 µL | 5 µL | 5 µL |
| Peak Incident Power | 280 W | 280 W | 280 W |
| Duty Factor | 13% | 9% | 11% |
| Cycles per Burst | 50 | 50 | 50 |
| Temperature | 10°C | 10°C | 10°C |
| Dithering | 3 mm Y at 20 mm/s | 3 mm Y at 20 mm/s | 1 mm Y at 20 mm/s |
| Repeat Process Duration | 10 sec | 10 sec | 10 sec |
| Number of Iterations | 6 | 6 | 6 |
| Delay Time | 10 sec | 10 sec | 10 sec |

5. Once created, select the "RNA Shearing" method from the drop-down menu to allow for the water bath temperature to equilibrate and reach set point.

### Thermocycler

Create the following 3 programs on a thermocycler:

| rRNA Depletion | | |
|---|---|---|
| **Step** | **Temperature** | **Time** |
| Lid Temperature | 85°C | N/A |
| Step 1 | 75°C | 2 min |
| Step 2 | 70°C | 2 min |
| Step 3 | 65°C | 2 min |
| Step 4 | 60°C | 2 min |
| Step 5 | 55°C | 2 min |
| Step 6 | 35°C | 2 min |
| Step 7 | 25°C | 2 min |
| Step 8 | 4°C | 2 min |
| Step 9 | 4°C | Indefinitely |

| cDNA Synthesis | | |
|---|---|---|
| **Step** | **Temperature** | **Time** |
| Lid Temperature | 80°C | N/A |
| Step 1 | 4°C | 1 min |
| Step 2 | 25°C | 5 min |
| Step 3 | 42°C | 90 min |
| Step 4 | 70°C | 10 min |
| Step 5 | 4°C | 1 min |
| Step 6 | 4°C | Indefinitely |

| Cycle Number Recommendations | |
|---|---|
| **RNA Input** | **Amplification Cycles** |
| 10 ng | 23 |
| 50 ng | 21 |
| 100 ng | 20 |
| 500 ng | 18 |

| Library Amplification | | | |
|---|---|---|---|
| **Step** | **Temperature** | **Time** | **Number of Cycles** |
| Lid Temperature | 98°C | N/A | N/A |
| Initial Denaturation | 98°C | 30 sec | 1 |
| Denature | 98°C | 5 sec | 18–23 (The cycle number will change depending on RNA input*) |
| Anneal | 55°C | 10 sec | |
| Extend | 72°C | 20 sec | |
| Final Extension | 72°C | 2 min | 1 |
| Hold | 4°C | 1 min | 1 |
| Hold | 4°C | Indefinitely | 1 |

## AFA-enabled Shearing of RNA

1. Thaw template RNA samples on ice.
2. Dilute RNA samples to desired concentration in nuclease-free water.

> **NOTE:** It is recommended to dilute to a concentration of at least 10 ng/µL.

3. Load 5 µL prepared RNA samples into wells of 8 AFA-TUBE TPX Strip and cover with 8 AFA-TUBE TPX Strip Caps.
4. Briefly centrifuge the 8 AFA-TUBE TPX Strip at 3,200xg for 1 min to ensure entire volume is at the bottom of the tubes.
5. Place the 8 AFA-TUBE TPX Strip with PSU Rack R230 8 AFA-TUBE TPX Strip into the Focused-ultrasonicator and start the AFA treatment described in Instrument Setup (***see Thermocycler Section***) to shear the RNA.
6. After program is finished, remove the plate or strip from the Focused-ultrasonicator, centrifuge at 3,200xg for 1 minute and store on ice or freeze at -20˚C until commencing with library prep (***see rRNA Depletion Section***).

## truCOVER RNA Library Prep

### rRNA Depletion

1. Thaw rRNA Depletion Mix, Hexamer RT Primer, Oligo dT Primer, 10X RT Buffer, and Nuclease Free Water at room temperature.
2. Vortex reagents and briefly centrifuge.
3. Dilute rRNA Depletion Mix to 0.1X (ex: 2 µL rRNA Depletion Mix + 18 µL Nuclease Free Water).
4. Prepare the rRNA Depletion Master Mix.

| Component | Volume for 1 Sample |
|---|---|
| 0.1X rRNA Depletion Mix | 1 µL |
| Hexamer RT Primer | 2 µL |
| Oligo dT Primer | 1 µL |
| 10X RT Buffer | 2 µL |

**Note:** When preparing the Master Mix for multiple samples, add 10% extra volume.

5. Add 6 µL of rRNA Depletion Master Mix to the 5 µL of sheared RNA in the 8 AFA-TUBE TPX Strip.

> **NOTE:** The final reaction volume must be 11 µL.

6. Attach strip cap to 8 AFA-TUBE TPX Strip, vortex to mix, and briefly centrifuge.
7. Incubate samples in a thermocycler using the "rRNA Depletion" program described in ***Instrument Setup Section***.

### cDNA Synthesis

1. Thaw DTT, dNTPs, and 10X $MgCl_2$ at room temperature.
2. Vortex reagents and briefly centrifuge before use.
3. Mix RNase Inhibitor and Reverse Transcriptase by gently flicking tubes and briefly centrifuge before storing on ice.
4. Add 2.5 µL Nuclease Free Water to each well of the Sample ID Primer plate. For example, if processing 8 samples, add 2.5 µL to 8 different wells.
5. Re-seal Sample ID Primer plate with strip caps, vortex to mix, and briefly centrifuge.
6. Incubate Sample ID Primer plate at room temperature for 10 minutes.
7. Prepare cDNA Synthesis Master Mix.

| Component | Volume for 1 Sample |
|---|---|
| DTT | 0.5 µL |
| dNTP | 2 µL |
| 10X $MgCl_2$ | 2 µL |
| Reverse Transcriptase | 1.5 µL |
| RNase Inhibitor | 0.5 µL |

**Note:** When preparing the Master Mix for multiple samples, add 10% extra volume.

8. Briefly centrifuge 8 AFA-TUBE TPX Strip containing rRNA-depleted RNA samples.
9. Add 2.5 µL rehydrated Sample ID Primer to RNA samples.
10. Add 6.5 µL cDNA Synthesis Master Mix to RNA samples.
11. Attach strip cap to 8 AFA-TUBE TPX Strip, vortex to mix, and briefly centrifuge.
12. Incubate samples in a thermocycler using the "cDNA Synthesis" program described in ***Instrument Setup Section***.

### cDNA Magnetic Bead Cleanup

1. Equilibrate Bead Purification Reagent and Elution Buffer (contained in **Box 2**) for 30 minutes at Room Temperature.
2. Prepare a sufficient volume of 80% ethanol for the number of samples to be purified (at least 800 µL per sample).
3. Vortex magnetic beads immediately before proceeding to the next step for at least 1 minute. Beads must be suspended homogeneously.
4. Add 33 µL Bead Purification Reagent and 11 µL nuclease-free water to the wells of the 8 AFA-TUBE TPX strip.
5. Mix samples by pipetting up and down 10 times or pulse-vortexing 10 times.
6. Incubate samples for 5 minutes at room temperature.
7. Place the tubes onto a magnetic separator and incubate for 2 minutes or until supernatant fully cleared of beads.
8. Remove and discard the supernatant. Do not remove samples from magnet.

9. Add 200 µL 80% ethanol.
10. Incubate for 30 seconds, then remove and discard supernatant.
11. Repeat ***Steps 9 & 10*** for a total of 2 washes.
12. Remove residual ethanol with a 10 µL pipette.
13. Allow the magnetic beads to air dry for 3 minutes.
14. Add 32 µL Elution Buffer and mix by pipetting up and down 10 times or pulse-vortexing 10 times.
15. Incubate for 4 minutes at room temperature.
16. Place the tubes onto a magnetic separator and incubate for 2 minutes or until supernatant fully cleared of beads.
17. Transfer 30 µL supernatant to fresh strip tubes.
18. Add 33 µL Bead Purification Reagent to all samples.
19. Perform a second clean-up by repeating *Steps 5–13*.
20. Add 25 µL Elution Buffer and mix by pipetting up and down 10 times or pulse-vortexing 10 times.
21. Incubate for 4 minutes at room temperature.
22. Place the tubes onto a magnetic separator and incubate for 2 minutes or until supernatant fully cleared of beads.
23. Transfer 23 µL supernatant to fresh strip tubes.

> **NOTE:** Samples may be stored at -20˚C before proceeding to Library Amplification.

### Library Amplification

1. Thaw a Covaris RNA UDI Index plate at room temperature.
2. Thaw Amplification Mix on ice.
3. Vortex and briefly centrifuge reagents before use.

> **NOTE:** If precipitate is observed in Amplification Mix, equilibrate to room temperature and vortex gently until dissolved completely.

4. Add 25 µL Amplification Mix to magnetic bead purified cDNA (***see cDNA Magnetic Bead Cleanup Section***).
5. Add 2 µL of a unique index contained in wells of the Covaris RNA UDI Index Set A plate to each cDNA sample.
6. Attach strip cap to PCR strip, vortex to mix, and briefly centrifuge.
7. Incubate samples in a thermocycler using the "Library Amplification" program described in Instrument Setup (***see Thermocycler Section***).

## Amplified Library Magnetic Bead Cleanup

1. Equilibrate Bead Purification Reagent to room temperature for 30 minutes before use.
2. Prepare a sufficient volume of 80% ethanol for the number of samples to be purified (at least 400 µL per sample).
3. Vortex magnetic beads immediately before proceeding to the next step for at least 1 minute. Beads must be suspended homogeneously.
4. Add 45 µL Bead Purification Reagent to all samples, and mix by pipetting up and down 10 times or pulse-vortexing 10 times.
5. Incubate samples for 5 minutes at room temperature.
6. Place the tubes onto a magnetic separator and incubate for 2 minutes or until supernatant fully cleared of beads.
7. Remove and discard the supernatant. Do not remove the tubes from the magnet.
8. Add 200 µL 80% ethanol.
9. Incubate for 30 seconds, then remove and discard supernatant.

10. Repeat ***Steps 8 & 9*** for a total of 2 washes.
11. Remove residual ethanol with a 10 µL pipette.
12. Allow the magnetic beads to air dry for 3 minutes.
13. Add 24 µL Elution Buffer and mix by pipetting up and down 10 times or pulse-vortexing 10 times.
14. Incubate for 4 minutes at room temperature.
15. Place the tubes onto a magnetic separator and incubate for 2 minutes or until supernatant fully cleared of beads.
16. Transfer 22 µL supernatant to fresh strip tubes.

    **Note:** Libraries are now ready for quality control (quantification, fragment size analysis), pooling and sequencing.

## Support and Technical Assistance

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

- **Telephone:**
  - **US & APAC:** +1 781.932.3959, during the hours of 8:30 a.m. to 5:00 p.m. EST, Monday through Friday
  - **EU:** +44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. GMT, Monday through Friday
- **E-mail:**
  - **US Customer Service:** customerservice@covaris.com
  - **EU/UK Customer Service:** emeacustomerservice@covaris.com
  - **APAC Customer Service:** APACcustomerservice@covaris.com
  - **Service and Instrumentation:** techsupport@covaris.com
  - **Applications:** applicationsupport@covaris.com



**US:** +1 781.932.3959
customerservice@covaris.com

**EU:** +44 (0)845 872 0100
emeacustomerservice@covaris.com

**APAC:** +86 137 6427 6714
APACcustomerservice@covaris.com

**Applications**
applicationsupport@covaris.com

**Service and Support**
techsupport@covaris.com

www.covaris.com | 2026© Covaris, LLC