truXTRAC® FFPE total Nucleic Acid Plus Kit – Magnetic Beads

The truXTRAC FFPE tNA Plus Magnetic Bead Kit (PN 520255) PK Solution and magnetic bead suspension should be stored at 2 to 8 °C upon arrival; other components at room temperature (RT) (15 to 25 °C).

Further Information

- truXTRAC FFPE tNA Plus Kit Manual (www.covaris.com/wp-content/uploads/pn_010451.pdf)
- Safety Data Sheets (www.covaris.com/resources/safety-data-sheets/)
- Application Support (ApplicationSupport@covaris.com)

Notes Before Starting

- Unless otherwise stated, perform all steps quickly at RT (15 to 25 °C)
- For initial preparation of reagents and methods, refer to the truXTRAC FFPE tNA Plus Kit Manual
- Set dry block heaters to 56 °C and 80 °C ± 3 °C, using technique recommended in the truXTRAC FFPE tNA Plus Kit Manual
- All centrifugation steps are done at room temperature (15 to 25 °C) unless otherwise stated
- DNase is not included in the truXTRAC FFPE tNA Plus Kit, however its use is highly recommended for RNA extraction
- Please refer to Kit Manual for more details

Paraffin Emulsification, Tissue Rehydration, and Lysis

1. Prepare Tissue Lysis Buffer (N samples: **440 µL** x N) with PK Solution (N samples: **44 µL** x N) and vortex for **3 seconds** or invert **10 times**
2. Load microTUBE-500 tubes with FFPE tissue
3. Add **440 µL** Tissue Lysis Buffer/PK Solution mix to each microTUBE-500
4. Cap microTUBE-500 tubes
5. **Process using "Acoustic Paraffin Emulsification" on the Covaris Focused-ultrasonicator**
6. After AFA processing, incubate samples in microTUBE-500 tubes at 56 °C for **30 minutes**
7. Remove microTUBE-500 tubes with heater adapters and let cool for **3 minutes**
8. Centrifuge tubes in adapters with barcode facing outward at 5,000 x g for **15 minutes**

Purification of RNA

1. Transfer **400 µL** of supernatant into a 2 mL microcentrifuge tube (***see manual for details***)
   - Save DNA-containing tissue pellet for DNA purification steps (***see manual for details***)
2. Incubate the 2 mL microcentrifuge tubes on heat block, previously verified so samples are at 80 °C, for ***20 minutes***
   - ***NOTE***: Heat block may need to be set above 80 °C
3. Remove microTUBE-500 tubes and cool at RT for **3 minutes**
4. Prepare Buffer BB3 (N samples: **1320 µL** x N) with Magnetic Bead Suspension (N samples: **8.8 µL** x N) and vortex for **3 seconds** or invert **10 times**
5. Add **1208 µL** of the BB3/Magnetic Bead Suspension mix to the RNA containing supernatant
6. Vortex for **10 seconds**
7. Incubate at 56 °C for **5 minutes**
8. Place microcentrifuge tubes on a magnetic stand and incubate for **5 minutes** or until all beads have been pulled to the magnet
9. Carefully remove and discard supernatant
10. Remove from magnetic stand
11. Add **1 mL** of Buffer WB4 and vortex for **10 seconds**
12. Repeat steps 8 through 10 to discard supernatant
13. Prepare 1X TURBO Master Mix (**96.8 µL** x N RNase-free H2O, **11 µL** x N 10X TURBO DNase Buffer, **2.2 µL** x N TURBO DNase) and add **100 µL** to each sample
14. Resuspend by pipetting **20 times**
15. Incubate for **30 minutes**
16. Add **300 µL** Buffer BB3 and vortex for **5 seconds**
17. Place tubes on magnetic stand and incubate for **5 minutes**, then remove all supernatant
18. Add **1 mL** of Buffer WB4 and vortex for **10 seconds**
19. Repeat steps 8 through 10 to discard supernatant
20. Add **1 mL** of 80% ethanol solution and vortex for **10 seconds**
21. Place microcentrifuge tubes on a magnetic stand and incubate for **2 minutes** or until all beads have been pulled to the magnet, then repeat steps 9 and 10
22. Add **300 µL** of 80% ethanol solution and vortex for **10 seconds**

23. Place microcentrifuge tubes on a magnetic stand and incubate for **2 minutes** or until all beads have been pulled to the magnet, then repeat steps 9 and 10 and ensure all supernatant is removed with a 20 uL pipette
24. Leave tubes uncapped, at RT, for **6 minutes**
25. Remove microcentrifuge tubes from magnetic stand and add **50 to 100 µL** of RNA Elution Buffer
26. Resuspend by pipette mixing **20 times**
27. Incubate at 56 °C for **5 minutes**
28. Transfer back to magnetic stand and incubate for **2 minutes**
29. Transfer eluate to a clean microcentrifuge tube for storage on ice before processing or -80 °C for long-term storage

Purification of DNA

1. Prepare Tissue Lysis Buffer (N samples: **352 µL** x N) with PK Solution (N samples: **88 µL** x N) and vortex for **3 seconds** or invert **10 times**
2. Add **400 µL** of Tissue Lysis Buffer/PK solution mix to the DNA-containing tissue pellet and re-cap
3. **Process using "Acoustic Pellet Resuspension" on the Covaris Focused-ultrasonicator**
4. After AFA processing, incubate samples in microTUBE-500 tubes for a minimum of **60 minutes** at 56 °C
5. Remove microTUBE-500 tubes and transfer directly to incubate at 80 °C for **60 minutes**
6. Let cool for **3 minutes** at ambient temperature
7. Transfer entire sample to 2 mL microcentrifuge tube
   - ***OPTIONAL***: Add **5 µL** of RNase A (10 mg/mL) solution and incubate for **5 minutes** at RT
8. Prepare BB3 Buffer (N samples: **792 µL** x N) with Magnetic Bead Suspension (N samples: **8.8 µL** x N) and vortex for **3 seconds** or invert **10 times**
9. Add **728 µL** of the BB3/Magnetic Bead Mix to each of the 2 mL microcentrifuge tubes
10. Vortex for **10 seconds**
11. Incubate at 56 °C for **5 minutes**
12. Place microcentrifuge tubes on a magnetic stand and incubate for **5 minutes** or until all beads have been pulled to the magnet
13. Carefully remove and discard supernatant
14. Remove from magnet stand

15. Add **1 mL** of Buffer WB3 and vortex for **10 seconds**
16. Repeat steps 12 through 14
17. Add **1 mL** of Buffer WB3 and vortex for **10 seconds**
18. Repeat steps 12 through 14
19. Add **1 mL** of 80% ethanol solution and vortex for **10 seconds**
20. Place microcentrifuge tubes on a magnetic stand and incubate for **2 minutes** or until all beads have been pulled to the magnet, then repeat step 12 through 14
21. Add **300 µL** of 80% ethanol solution and vortex for **10 seconds**
22. Place microcentrifuge tubes on a magnetic stand and incubate for **2 minutes** or until all beads have been pulled to the magnet and ensure all supernatant is removed with a 20 µL pipette
23. Leave tubes uncapped for **6 minutes**
24. Remove microcentrifuge tubes from magnetic stand and add **50 to 100 µL** of Buffer BE
25. Resuspend by pipette mixing **20 times**
26. Incubate at 56 °C for **5 minutes**
27. Transfer back to magnetic stand and incubate for **2 minutes**
28. Transfer eluate to a clean microcentrifuge tube for processing, store at 2 to 8°C for short-term storage, or -20 °C for long-term storage

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