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

- 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, 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)
   - Save DNA-containing tissue pellet for DNA purification steps (see manual)
2. Incubate the 2 mL microcentrifuge tubes on heat block, previously verified so samples are at 80 °C, for 30 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 10 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 10 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 (93.5 µL x N RNase-free H2O, 11 µL x N 10X TURBO DNase Buffer, 5.5 µL x N TURBO DNase) and add 100 µL to each sample
14. Resuspend by pipetting 20 times
15. Incubate at room temperature for 30 minutes
16. Add 300 µL Buffer BB3 and vortex for 5 seconds
17. Incubate at RT for 10 minutes
18. Place tubes on magnetic stand and incubate for 5 min., then remove all supernatant
19. Add 1 mL of Buffer WB4 and vortex for 10 seconds
20. Repeat steps 8 through 10 to discard supernatant
21. Add 1 mL 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, then repeat steps 9 and 10
23. Add 300 µL of 80% ethanol solution and vortex for 10 seconds
24. 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 µL pipette
25. Leave tubes uncapped, at RT, for **6 minutes**
26. Remove microcentrifuge tubes from magnetic stand and add **50 to 100 µL** of RNA Elution Buffer
27. Resuspend by pipette mixing **20 times**
28. Incubate at 56 °C for **5 minutes**
29. Transfer back to magnetic stand and incubate for **2 minutes**
30. 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 a 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 **10 seconds** or invert **10 times**
9. Add **728 µL** of the BB3/Magnetic Bead Mix to each 2 mL microcentrifuge tube
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

Notes

_______________________________________________________________________________________________________________________
_______________________________________________________________________________________________________________________
_______________________________________________________________________________________________________________________
_______________________________________________________________________________________________________________________
_______________________________________________________________________________________________________________________
_______________________________________________________________________________________________________________________