truXTRAC® FFPE total Nucleic Acid Plus Kit – Column

The truXTRAC FFPE tNA Plus Column Kit (PN 520252) PK solution 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 (https://www.covaris.com/wp/wp-content/uploads/2020/07/pn_010489.pdf)
- Safety Data Sheets (www.covaris.com/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 Column Kit Manual
- Set dry block heaters to 56 °C and 80 °C ± 3 °C, using technique recommended in the truXTRAC Column 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 centrifuge/heat block 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. Add **375 µL** of Total NA Plus B1 Buffer & mix by vortexing for **3 seconds**
5. Add **350 µL** 100% isopropanol & mix by vortexing for **3 seconds**
6. Transfer **600 µL** to RNA purification column and centrifuge at 11,000 x g for **30 seconds**
7. Discard flow-through, place column back into Collection Tube and repeat step 6 until all sample material has passed through column
8. Add **650 µL** of RNA Wash Buffer to the RNA Purification Column
9. Centrifuge at 11,000 x g for **30 seconds** and discard flow-through
10. 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
11. Incubate for **30 minutes**
12. Sequentially add **175** B1 Buffer and then **300 µL** 65% isopropanol
13. Close cap & vortex to mix
14. Centrifuge at 11,000 x g for **30 seconds**
15. Pipette flow-through back into column & repeat step 13
16. Add **650 µL** of RNA Wash Buffer to the RNA Purification Column
17. Centrifuge at 11,000 x g for **30 seconds**
18. Discard flow-through and put column back into collection tube
19. Centrifuge at 16,000 x g for **1 minute**
20. Place column in a new RNA Elution Tube (1.5 mL) and add **30 µL** (for high concentration) or **50 µL** (for high yield) RNA Elution Buffer to the center of the column
    - May be repeated for more yield, please refer to the truXTRAC Column Kit Manual
21. Incubate for **2 minutes**
22. Centrifuge at 16,000 x g for **1 minute**
22. Remove the column from the RNA Elution Tube and retain
23. Keep the RNA eluate on ice for further processing or store at -80 °C long-term

Purification of DNA

1. Aliquot **110 µL** of BE Buffer per sample in a 1.5 mL microcentrifuge tube
2. Preheat BE Buffer to 80 °C until needed for elution from the column
3. 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**
4. Add **400 µL** of Tissue Lysis Buffer/Proteinase K mix to the DNA-containing tissue pellet and re-cap
5. **Process using "Acoustic Pellet Resuspension" on the Covaris Focused-ultrasonicator**
6. After AFA processing, incubate samples in microTUBE-500 tubes for a minimum of **60 minutes** at 56 °C
7. Remove microTUBE-500 tubes and transfer directly to incubate for **60 minutes** at 80 °C
8. Let cool for **3 minutes** at RT
9. Transfer entire sample to 2 mL microcentrifuge tube
   - ***OPTIONAL***: RNase treatment; Add **5 µL** of RNase A (**10 mg/mL**) solution and incubate for **5 minutes** at RT
10. Add **560 µL** of Total NA Plus B1 Buffer and vortex for **3 seconds**
11. Add **640 µL** of 100% ethanol and vortex for **3 seconds**
12. Transfer **600 µL** to a DNA Purification Column in a Collection Tube, centrifuge at 11,000 x g for **1 minute** and discard flow-through
13. Repeat step 12 until all sample material has passed through column
14. Add **500 µL** of BW Buffer to the DNA Purification Column
15. Centrifuge at 11,000 x g for **1 minute** and discard flow-through
16. Add **600 µL** of B5 Buffer to the DNA Purification Column
17. Centrifuge at 11,000 x g for **1 minute** and discard flow-through
18. Centrifuge at 16,000 x g for **1 minute**
19. Place column into a clean 1.5 mL microcentrifuge tube
20. Add **50 µL** of pre-warmed BE Buffer to the center of the column and incubate at ambient temperature for **3 minutes**
21. Centrifuge at 11,000 x g for **1 minute**
22. Repeat steps 20 and 21
    - Additional elution steps may be added, refer to the truXTRAC Column Kit Manual for details
23. Remove the Column from the microcentrifuge tube and retain tube containing DNA eluate, 2 to 8°C for short-term storage or -20°C for long-term storage

Notes:

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