Covaris®

truXTRAC® FFPE total NA Ultra Kit – Magnetic Bead (25)

Adaptive Focused Acoustics® (AFA®)-based Sequential RNA and DNA Extraction from FFPE Tissues on the KingFisher™ Duo Prime Purification System

PN 520304

Part Number: 010527 Rev B | November 2020

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General Information

Intended Use

The truXTRAC FFPE total NA (tNA) Ultra Kit - Magnetic Bead (PN 520304) is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of any disease.

Introduction

The truXTRAC FFPE total NA (tNA) Ultra Kit - Magnetic Bead is designed for efficient and sequential extraction of total nucleic acids (RNA and DNA) from Formalin Fixed, Paraffin Embedded (FFPE) tissue samples using Covaris Adaptive Focused Acoustics (AFA).

AFA-energetics® enables the active removal of paraffin from FFPE tissue samples in an aqueous buffer, while simultaneously rehydrating the tissue. Compared to traditional passive, chemical-based methods of paraffin removal, this non-contact mechanical process is more efficient as the paraffin is removed and emulsified from the tissue. Uniquely, AFA enables increased yields of nucleic acids and minimizes the degradation of nucleic acids exposed at the FFPE section surface. The truXTRAC protocol results in high yields of high-quality RNA and DNA for sensitive analytical methods such as next-generation sequencing (NGS) or qPCR/RT-qPCR.

This protocol is optimized for up to 20 µm of total FFPE tissue sections. For microdissection samples, please contact ApplicationSupport@covaris.com.

The protocol enables automated, sequential purification of RNA and DNA from up to 12 FFPE samples at a time. In this protocol, RNA and DNA are purified separately using two 96-well plates (one for RNA and one for DNA). The KingFisher BindIT Software runs specific BindIT protocol files for each of the following purification methods: 1) RNA purification, 2) RNA Purification with DNase treatment, and 3) DNA Purification. Only one RNA purification BindIT protocol file and one DNA purification BindIT protocol file is used during purification from FFPE tissues.

Important Notes on FFPE Samples:

The yield of DNA and RNA from FFPE tissue blocks is highly variable. Factors such as fixation time, size and thickness of the cores, the ratio of tissue to paraffin, the type of tissue, and the age and storage conditions of the FFPE block are the main causes for this variability.

More importantly, however, the quality of DNA and RNA isolated from FFPE samples can also be highly variable. During the fixation process, DNA and RNA are cross-linked to proteins and other nucleic acid molecules to varying degrees. The nucleic acid fragment or strand length isolated from FFPE samples is generally shorter as compared to nucleic acids that are isolated from fresh or frozen tissues [1]. This is particularly evident in older FFPE sample blocks or sample blocks stored at elevated temperatures. Thus, an advanced mechanical deparaffinization process is important to extract the high quality nucleic acids required for sensitive analytical techniques. Covaris AFA enables non-contact mechanical removal of paraffin from FFPE samples to improve the yield and quality of extracted nucleic acids.

Note for Users:

If you require any assistance with this product please refer to Troubleshooting (Appendix) in this protocol, check the FAQs found on our website, or contact Covaris Application Support at ApplicationSupport@covaris.com.

Revision History

Part Number	Revision	Date	Description of Change
010527	A	06/2020	Initial Release

Kit Contents

- Tissue Lysis Buffer..... 25 ml
- Proteinase K (PK Solution)..... 3.5 ml
- Magnetic Bead Suspension ..... 0.5 ml
- Buffer BB3..... 45 ml
- Buffer WB3 ..... 2 x 60 ml
- RNA Elution Buffer ..... 3.5 ml
- Buffer BE ..... 7.5 ml
- AFA-TUBE PP Screw-Cap 0.5 ml (PN 520301).... 25

SDS infrmation is available at: www.covaris.com/resources/safety-data-sheets/

Storage

Upon kit arrival, store the Proteinase K solution and Magnetic Bead Suspension at 2 °C to 8 °C. Store all other kit components at room temperature.

KingFisher Duo Prime BindIT Protocol Files

- 520304_FFPE_Ultra_RNA_Duo_Protocol.bdz
- 520304_FFPE_Ultra_RNA_DNase_Duo_Protocol.bdz
- 520304_FFPE_Ultra_DNA_Duo_Protocol.bdz

Laboratory Equipment, Chemicals, and Consumables to be Supplied by User

Required Laboratory Equipment and Accessories

- KingFisher Duo Prime Purification System (Thermo Fisher Scientific, PN 5400110)
- 0.5 ml Centrifuge Adapters (Eppendorf, PN 022636227)
- Dry block heater with blocks to accommodate 2 ml tubes or temperature-controlled water bath able to accurately heat between 50 °C to 90 °C
- Dry block heater with blocks to accommodate 0.5 ml microcentrifuge conical tubes or temperature-controlled water bath able to accurately heat between 50 °C to 90 °C

Required Chemicals and Enzymes

- 100% ethanol, molecular biology grade (e.g., AmericanBio, PN AB00515)
- Nuclease-free water (e.g., Invitrogen, PN AM9930)

Optional Enzymes

- TURBO DNase (2 U/µL) (Thermo Fisher Scientific, PN AM2238)
- DNase-free RNase A (10 mg/ml) (e.g., Thermo Fisher Scientific, PN EN0531)

Required Consumables

- KingFisher Duo Pack for 96 deep well plate, includes tip combs, plate and elution strips for 96 samples (Thermo Fisher, PN 97003530) or see below to purchase separately;
  - Microtiter deep well 96 plate (Thermo Fisher Scientific, PN 95040460)
  - KingFisher Duo 12-tip comb (Thermo Fisher Scientific, PN 97003500)
  - KingFisher Duo elution strip (Thermo Fisher Scientific, PN 97003520)
  - KingFisher Duo cap for elution strip (Thermo Fisher Scientific, PN 97003540)
- Eppendorf tubes 2 ml (Eppendorf, PN 0022363344)
- Eppendorf tubes 5 ml (Eppendorf, PN 0030119401)
- Tube to make Binding Buffer/Bead Mixes (15 ml or 50 ml tubes)

Covaris Focused-ultrasonicator Accessories and Plate Definitions

The table below contains the parts and plate definitions necessary to run the protocol. Use the parts and plate definitions specific to your Covaris Focused-ultrasonicator.

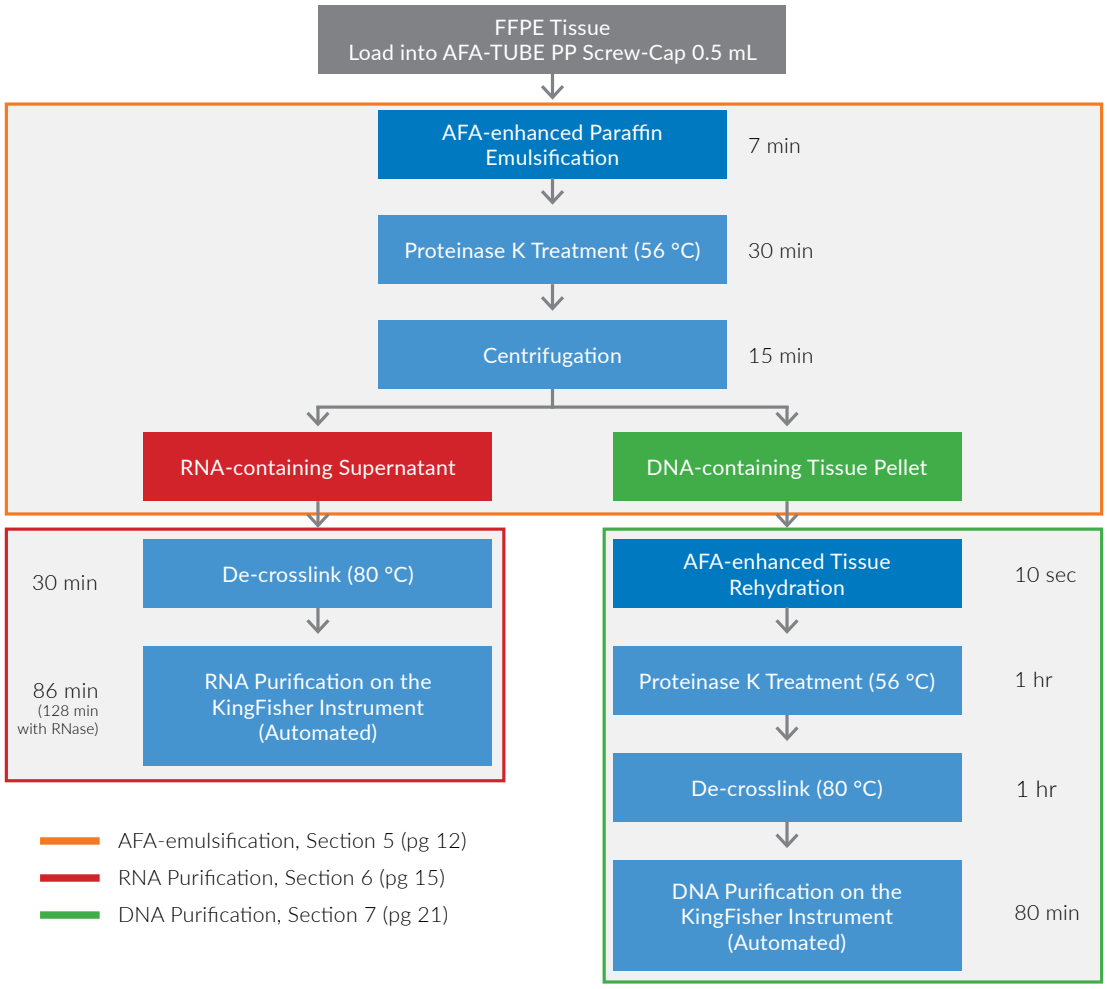
Instrument	ML230	M220
Holder/Rack Description (PN)	Rack 6 Place AFA-TUBE PP Screw-Cap 0.5 ml (PN 500696)	Holder XTU (500414)
Required Accessories (PN)	N/A	XTU Insert (PN 500692)
Plate definition file name	<500696 Rack 6 Place AFA-TUBE PP Screw-Cap 0.5 ml>	N/A

FFPE tNA Extraction and Purification Workflow

Using the Adaptive Focused Acoustics (AFA) process, FFPE tissue samples are prepared in Tissue Lysis Buffer in the presence of Proteinase K, followed by an incubation at 56 °C for a short duration. This results in the release of RNA while minimizing over-digestion of the tissue and loss of genomic DNA.

The RNA-containing supernatant is separated from the DNA-containing tissue by a centrifugation step. RNA is then de-crosslinked and purified using the KingFisher.

Sequentially, DNA is released from the DNA-containing tissue by AFA-enhanced Proteinase K digestion, following a de-crosslinking step. DNA is purified using the KingFisher.



1 - FFPE Sample Input Requirements and Guidelines

The truXTRAC protocol is highly efficient at mechanically removing paraffin, while simultaneously rehydrating the tissue.

> **CAUTION:** Do NOT exceed the input requirements in the tables below. Overloading will negatively impact the quality and quantity of extractable nucleic acids.

Slide Section Input Requirements:

Slide Collection Method	Maximum Input per microTUBE-500
Scalpel or razor blade to scrape material from slides	20 µm of total thickness Area: 100 mm^2 (4 slides at 5 µm thick = 20 µm total thickness)

Curls/Scrolls Input Requirements:

For best results, minimize the amount of wax present by trimming. We recommend no more than 1-part wax to 1-part tissue.

FFPE Curl/Scroll Thickness	Maximum Scrolls per AFA-TUBE PP Screw-Cap 0.5 ml
5 µm	4
10 µm	2
15 µm	1

2 - Preparation of Reagents

Follow these instructions before starting the FFPE tNA isolation protocol.

1. ***Tissue Lysis Buffer***: Check this buffer visually for a white precipitate that may form during storage. If white precipitate is visible, incubate the buffer bottles at 50 °C to 60 °C for 5 to 10 minutes before use to dissolve any precipitate.
2. ***80% ethanol***: Prepare 80% ethanol by mixing 4 parts 100% ethanol with 1 part nuclease free water. One sample requires 2.2 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 2.6 ml.

3 - Preparation of Heat Blocks

1. Preheat dry block heaters to 56 °C and 80 °C ± 3 °C. It is critical that these temperatures are accurate in order to successfully execute the protocol.
2. Test the temperature of your heat blocks:
   a. Place a 0.5 ml microcentrifuge tube filled with water into the heat block for 0.5 ml tubes, and a 2 ml microcentrifuge tube filled with water into the heat block for 2 ml tubes.
   b. Immerse a thermometer into the tube.
   c. Wait until the temperature has reached the plateau.
   d. Adjust the Set-temperature accordingly until the temperature inside the microcentrifuge tube has reached 56 °C ± 2 °C or 80 °C ± 2 °C.

> **CAUTION:** The AFA-TUBE PP Screw-Cap 0.5 ml must be used in conjunction with a compatible heat block, such as, Eppendorf SmartBlock™ 0.5 mL, thermoblock for 24 reaction vessels 0.5 mL, incl. Transfer Rack 0.5 mL (Eppendorf, Cat No. 5361000031).
>
> It is important to use an accurate heating source for incubation of AFA-TUBE PP Screw-Cap 0.5 ml and microcentrifuge tubes during Proteinase K and de-crosslinking incubations. Deviation from the indicated temperatures can adversely impact quality and quantity of purified nucleic acids.

4 - Focused-ultrasonicator Setup

For detailed instructions on how to prepare and use your instrument, please refer to the respective Covaris User Manual. If you do not see a Plate Definition on your system, please contact Covaris Technical Support (techsupport@covaris.com)

Refer to ***Page 4*** for Plate Definitions and required Focused-ultrasonicator accessories.

1. **Create "Acoustic Paraffin Emulsification" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "Acoustic Paraffin Emulsification" using the Covaris SonoLab method editor. Save the program for later use.

Instrument	ML230	M220
Peak Incident Power (PIP) (Watt)	330	50
Duty Factor (%)	30	15
Cycles Per Burst (CPB)	1000	800
Treatment time (seconds)	480	300
Bath temperature (°C)	20	20
Water Level (run)	Automatic	Full

2. **Create "Acoustic Pellet Resuspension" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "Acoustic Pellet Resuspension" using the Covaris SonoLab method editor. Save the program for later use.

Instrument	ML230	M220
Peak Incident Power (PIP) (Watt)	330	50
Duty Factor (%)	30	15
Cycles Per Burst (CPB)	1000	800
Treatment time (seconds)	10	300
Bath temperature (°C)	20	20
Water Level (run)	Automatic	Full

5 - Paraffin Emulsification, Tissue Rehydration, and Lysis

1. Prepare Lysis Buffer/Proteinase K Mix by following instructions in Table 1 below and mix by inverting 10 times or vortexing for 3 seconds.

> **CAUTION:** The Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min of preparation.

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	510 µl	510 µl x N
Proteinase K Solution	40 µl	40 µl x N

Table 1. Tissue Lysis Buffer /Proteinase K Mix. *Calculation includes 10% excess in final volume

2. Open the AFA-TUBE PP Screw-Cap 0.5 ml and load the FFPE tissue to the bottom of the tube.
3. Add 500 µl Tissue Lysis Buffer/Proteinase K Mix to AFA-TUBE PP Screw-Cap 0.5 ml.
4. Make sure that the tissue is completely submerged in Tissue Lysis Buffer/Proteinase K Mix.
5. Close the AFA-TUBE PP Screw-Cap 0.5 ml tightly with the Screw-Cap and transfer the AFA-TUBE PP Screw-Cap 0.5 ml to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the microTUBE(s) into the Focused-ultrasonicator for processing.

> **CAUTION:** If treating less than 6 samples on ML230, load AFA-TUBE PP Screw-Cap 0.5 ml containing water in the remaining rack positions. Screw-Cap 0.5 ml into the ML230 Focused-ultrasonicator for processing to prevent splashing.

6. Process the sample using the "***Acoustic Paraffin Emulsification***" program on the Focused-ultrasonicator.

Before AFA *After AFA*

> **NOTE:** It is expected that the solution will turn milky white.

7. Remove the AFA-TUBE PP Screw-Cap 0.5 ml from the Focused-ultrasonicator and load the tube into the 0.5 ml Heat Block. When processing in batches, samples may be kept at room temperature for up to two hours prior to Proteinase K incubation at 56 °C (Step 7).
8. Incubate for 30 minutes at 56 °C. Remove AFA-TUBE PP Screw-Cap 0.5 ml and let it cool at room temperature for 3 min.

> **CAUTION:** Do not chill on ice as rapid cooling will cause detergents to precipitate.

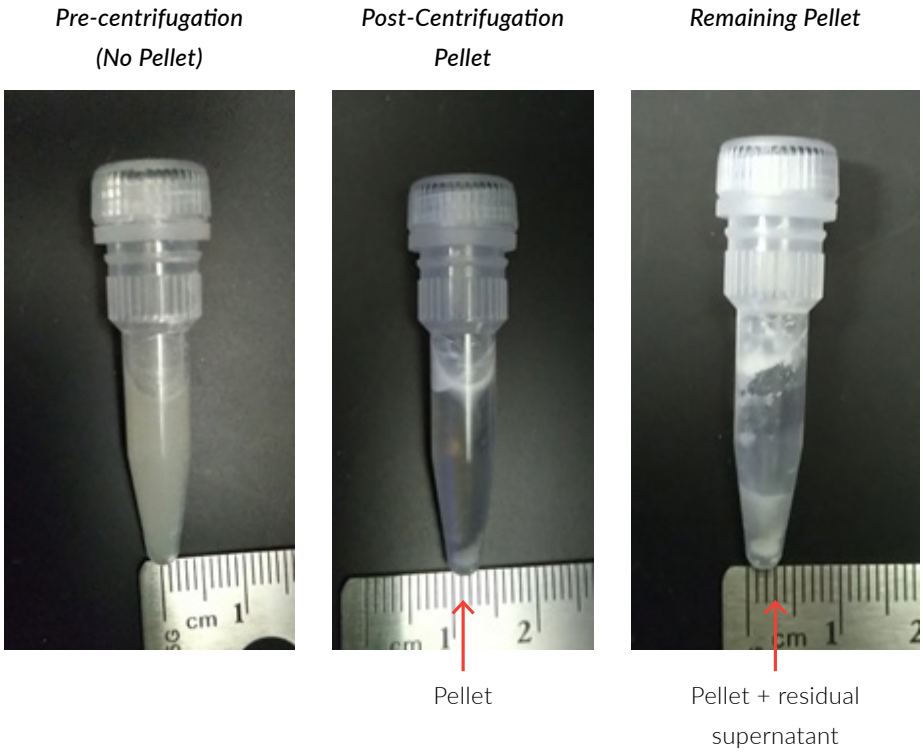
9. Place AFA-TUBE PP Screw-Cap 0.5 ml with 0.5 ml tube centrifuge adapters into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 15 minutes.

> **NOTE:** The centrifuge lid may not close and may need to be left off during centrifugation.

10. Open the AFA-TUBE PP Screw-Cap 0.5 ml and carefully transfer 450 µl RNA supernatant into a 2 ml microcentrifuge tube.

CAUTION: This is a critical step in the workflow. By following these guidelines, the risk of losing the DNA-containing tissue pellet will be minimized:

a. Locate the DNA-containing tissue pellet. It will be located at the bottom of the tube with more pellet toward the side faces outward during centrifugation. The pellet may appear faint and difficult to see.
b. Tilt the tube slightly away from the pellet.
c. Using a 200 µl pipette with a 200 µl pipette tip, slowly pierce the upper emulsified wax layer and carefully aspirate the supernatant while simultaneously lowering the tip following the liquid level. Place the pipette tip towards the tube wall that faces away from the pellet. DO NOT USE WIDE-MOUTH TIPS.
d. A layer of emulsified wax may descend obscuring the pellet. This is normal. Leave approximately 50 µl of supernatant behind. This will not significantly impact RNA or DNA yield. significantly



NOTE: If the pellet becomes dislodged from the bottom of the AFA-TUBE PP Screw-Cap 0.5 ml before the RNA supernatant has been removed, repeat centrifugation (Step 8) to re-pellet the DNA. Remove RNA supernatant as described in Step 10.

11. Save the DNA-containing tissue pellet for subsequent DNA purification as described in Section-8. Proceed immediately to RNA Purification (Section-6 or Section-7).

    The DNA-containing pellet can be stored on ice or at 2 °C to 8 °C for up to 1 day. For longer periods, store at –15 °C to –30 °C.

6 - FFPE RNA Purification without DNase Treatment on the KingFisher

The protocol allows purification of RNA from 1 to 12 FFPE samples at a time without DNase treatment. For DNase treatment of RNA samples, follow the procedure outlined in Section 7.

1. Set up the 2 ml dry-heat block heaters as explained in Section-3 to 56 °C (Step 6) and 80 °C (Step 2).
2. Incubate 2 ml microcentrifuge tube with the RNA-containing supernatant at 80 °C for 30 minutes. Remove tubes from the heat block and cool at room temperature for 3 minutes.
3. Prepare BB3/Magnetic Bead Mix according to Table 2 below.

Reagent	Volume for one sample*	Volume for N samples*
BB3	607 µl	607 µl x N
Magnetic Bead Suspension	8.8 µl	8.8 µl x N

Table 2. BB3/Magnetic Bead Mix For RNA. *Calculation includes 10% excess in final volume

> **CAUTION:** Thoroughly vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using.

4. Add 550 µl of BB3/Magnetic Bead Mix to the RNA-containing supernatant and cap the microcentrifuge tube.
5. Vortex the microcentrifuge tube for 5 seconds.
6. Incubate the microcentrifuge tube at 56 °C for 5 minutes.
7. Pipette each of the samples into a well of Row A of the 96 Deep Well KingFisher Plate.

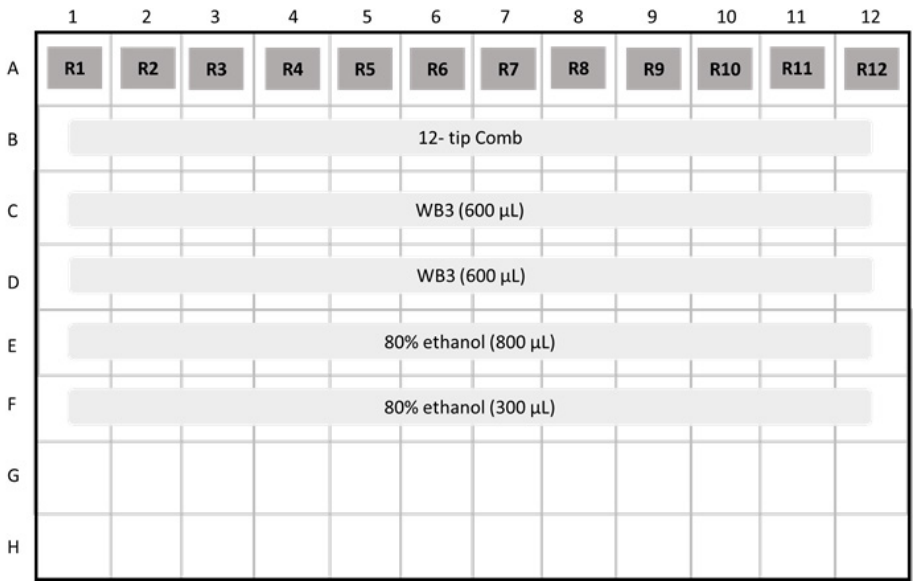


Figure 1. RNA Plate Layout.

8. Set up the remainder of the RNA Plate as shown in Figure 1. (Use as many wells as needed per the number of samples.)
   a) Add the 12-tip comb to the wells of Row B.
   b) Add 600 µl of WB3 into Row C (wells C1 to C12).
   c) Add 600 µl of WB3 into Row D (wells D1 to D12).
   d) Add 800 µl of 80% ethanol into Row E (wells E1 to E12).
   e) Add 300 µl of 80% ethanol into Row F (wells F1 to F12).
   f) Add 50 µl of RNA Elution Buffer into wells 1 to 12 in a separate elution strip.

> **CAUTION:** In order to minimize evaporation of ethanol, it is highly recommended to proceed with the purification on the KingFisher immediately after filling the plate.

9. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:
    - 520304_FFPE_Ultra_RNA_Duo_Protocol.bdz

> **CAUTION:** Do not load the plate before pressing "Play".

10. Press "Play" and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
11. Close the front lid while the KingFisher is running.
12. After the run is complete, a final prompt will appear: "Unload RNA Plate and RNA Elution Strip". Press the "Check Mark", unload the RNA Plate and cap the elution strip containing the RNA. Place the elution strip immediately on ice or transfer the eluted RNA into clean microcentrifuge tubes.

> **NOTE:** It is normal for the final elution volume to be 10-15% less than the input due to loss during instrument run.

Store the eluted RNA on ice until further processing. For longer term, store the RNA at -80 °C.

7 - FFPE RNA Purification with DNase Treatment on the KingFisher

The protocol allows purification of RNA from 1 to 12 FFPE samples at a time with DNase treatment.

1. Set up the 2 ml dry-heat block heaters as explained in Section-3 to 56 °C (Step 6) and 80 °C (Step 2).
2. Incubate 2 ml microcentrifuge tube with the RNA-containing supernatant at 80 °C for 30 minutes. Remove tubes from the heat block and cool at room temperature for 3 minutes. A schematic plate map for RNA purification on the KingFisher Duo Prime Purification System is shown in Figure 2.
3. Prepare BB3/Magnetic Bead Mix according to Table 3 below.

Reagent	Volume for one sample*	Volume for N samples*
BB3	607 µl	607 µl x N
Magnetic Bead Suspension	8.8 µl	8.8 µl x N

Table 3. BB3/Magnetic Bead Mix For RNA. *Calculation includes 10% excess in final volume

> **CAUTION:** Thoroughly vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using.

4. Add 550 µl of BB3/Magnetic Bead Mix to the RNA-containing supernatant and cap the microcentrifuge tube.
5. Vortex the microcentrifuge tube for 5 seconds.
6. Incubate the microcentrifuge tube at 56 °C for 5 minutes.
7. Pipette each of the samples into Row A of a deep well 96 plate , following the layout in Figure 2.

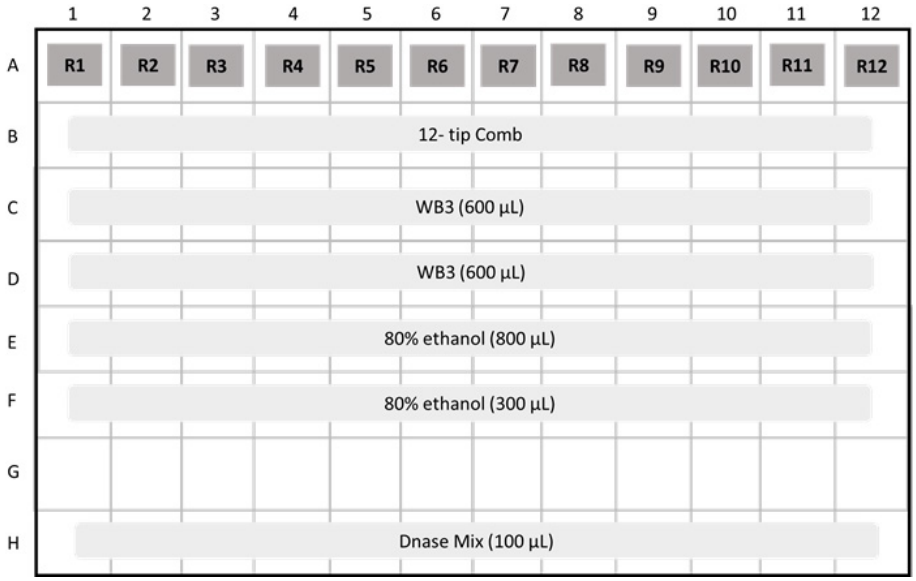


Figure 2. RNA with DNase Plate Layout.

8. Prepare DNase Mix according to Table 4 below in a 2 ml tube. Mix gently by inversion.

Reagent	Volume for one sample*	Volume for N samples*
Nuclease-free Water	93.5 µl	93.5 µl x N
10X DNase Buffer	11 µl	11 µl x N
TURBO DNase	5.5 µl	5.5 µl x N

Table 4. DNase Mix. *Calculation includes 10% excess in final volume

9. Set up the remainder of the RNA Plate as shown in Figure 2. (Use as many wells as needed per the number of samples.)
   a) Add the 12-tip comb to Row B.
   b) Add 600 µl of WB3 into Row C (wells C1 to C12.)
   c) Add 600 µl of WB3 into Row D (wells D1 to D12.)
   d) Add 800 µl of 80% ethanol into Row E (wells E1 to E12.)
   e) Add 300 µl of 80% ethanol into Row F (wells F1 to F12.)
   f) Add 100 µl of DNase mix into Row H (wells H1 to H12.)
   g) Add 50 µl of RNA Elution Buffer into wells 1 through 12 in a separate elution strip.

> **CAUTION:** In order to minimize evaporation of ethanol and ensure optimal activity of the DNase, it is highly recommended to proceed with the purification on the KingFisher immediately after filling the plate.

10. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:
    - 520304_FFPE_Ultra_RNA_DNase_Duo_Protocol.bdz

> **CAUTION:** Do not load the plate before pressing "Play".

11. Press “Play” and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
12. Close the front lid while the KingFisher is running.
13. After 1 hour and 27 minutes, the instrument will prompt a message to remove the RNA Plate.
    a) Remove the RNA Plate from the KingFisher.
    b) Add 200 µl of BB3 into Row H (wells H1 through H12).
    c) Place the RNA Plate back into the KingFisher.
    d) Press the “Check Mark”.
14. After the run is complete, a final prompt will appear: “Unload RNA Plate and RNA Elution Strip”. Press the “Check Mark”, unload the RNA Plate from the instrument, and cap the elution strip containing the RNA. Place the elution strip immediately on ice or transfer the eluted RNA into clean microcentrifuge tubes.

> **NOTE:** It is normal for the final elution volume to be 10-15% less than the input due to loss during instrument run.

Store the eluted RNA on ice until further processing. For longer term, store the RNA at -80 °C.

8 -DNA Purification on the KingFisher

1. Preheat dry block heaters with 0.5 ml heat blocks to 56 °C (Steps 7 and 15) and 80 °C (Step 9) ± 2C.
2. Prepare Tissue Lysis Buffer/Proteinase K Mix DNA in a microcentrifuge tube following instructions in Table 5 and mix by inverting 10 times or vortexing for 3 seconds.

> **CAUTION:** The Tissue Lysis Buffer/Proteinase K Mix should be stored at room temperature and used within 30 min after preparation.

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	352 µl	352 µl x N
Proteinase K Solution	88 µl	88 µl x N

Table 5. Tissue Lysis Buffer/Proteinase K Mix for DNA. *Calculation includes 10% excess in final volume

3. Open the AFA-TUBE PP Screw-Cap 0.5 ml with the DNA-containing tissue pellet and add 400 µl of the Tissue Lysis Buffer/Proteinase K Mix for DNA.
4. Close the AFA-TUBE PP Screw-Cap 0.5 ml tightly with the Screw-Cap and transfer the AFA-TUBE PP Screw-Cap 0.5 ml to the appropriate rack or holder/insert for your Focused-ultrasonicator. Load the rack or holder/insert containing the AFA-TUBE PP Screw-Cap 0.5 ml into the Focused-ultrasonicator for processing. If treating less than 6 tubes per treatment, insert tubes containing water in the remaining rack positions.

> **CAUTION:** If treating less than 6 samples on ML230, load AFA-TUBE PP Screw-Cap 0.5 ml containing water in the remaining rack positions to prevent splashing.

5. Process the sample using the “***Acoustic Pellet Resuspension***” program on your Focused-ultrasonicator.
6. Remove the AFA-TUBE PP Screw-Cap 0.5 ml from the Focused-ultrasonicator and load it into the 0.5 ml heat block set to 56 °C.
7. Incubate for a minimum of 60 minutes at 56 °C.

> **NOTE:** The Proteinase K-treated sample can be stored at room temperature for up to an additional hour. Do not chill on ice.

8. Remove AFA-TUBE PP Screw-Cap 0.5 ml from the heat block and transfer directly to dry heat block set-up for 80 °C incubation.
9. Incubate for 60 minutes at 80 °C.
10. Remove AFA-TUBE PP Screw-Cap 0.5 ml from the heat block and let cool for 3 minutes at room temperature.
11. Transfer the entire sample to a clean 2 ml microcentrifuge tube.
    Optional RNA removal step: At this point the sample can be treated with RNase A to remove residual RNA before continuing with DNA purification. Add 5 µl of RNase A (10 mg/ml) solution and incubate for 5 minutes at room temperature, then continue to step 12.
12. Prepare BB3/Magnetic Bead Mix according to Table 6 below.

Reagent	Volume for one sample*	Volume for N samples*
BB3	607 µl	792 µl x N
Magnetic Bead Suspension	8.8 µl	8.8 µl x N

Table 6. BB3/Magnetic Bead Mix For DNA. *Calculation includes 10% excess in final volume

> **CAUTION:** Thoroughly vortex the Magnetic Bead Suspension and BB3/Magnetic Bead Mix before using.

13. Add 550 µl of BB3/Magnetic Bead Mix to the DNA containing supernatant and cap the microcentrifuge tube.
14. Vortex the microcentrifuge tube for 5 seconds.
15. Incubate the microcentrifuge tube at 56 °C for 5 minutes.
16. Pipette each of the samples into Row A of the KingFisher Plate, following the layout in Figure 3.

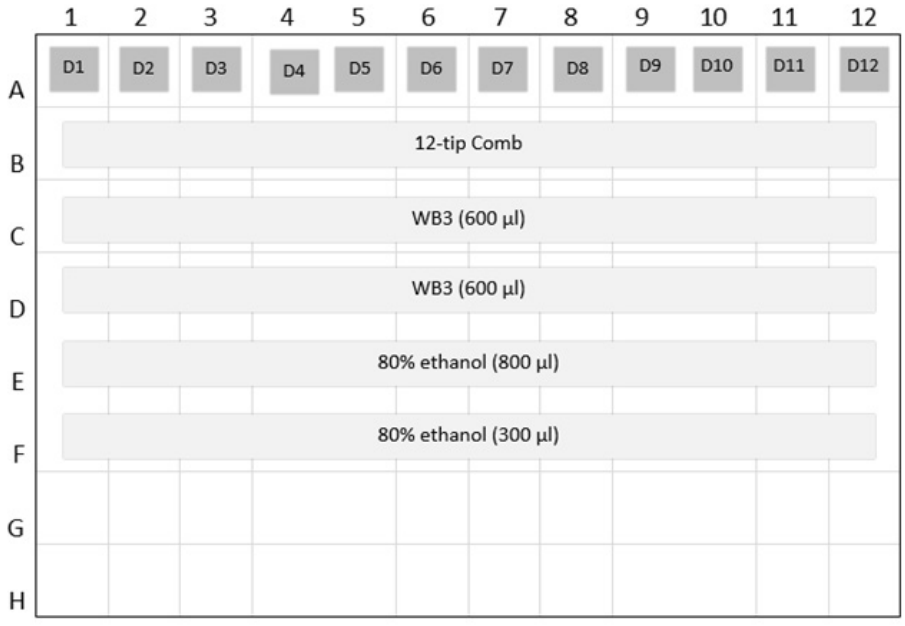


Figure 3. DNA Plate Layout.

17. Set up the remainder of the DNA Plate as shown in Figure 3. (Use as many wells as needed per number of samples.)
    a) Add the 12-tip comb to Row B.
    b) Add 600 µl of WB3 into Row C (wells C1 to C12.)
    c) Add 600 µl of WB3 into Row D (wells E1 to E12.)
    d) Add 800 µl of 80% ethanol into Row E (wells E1 to E12.)
    e) Add 300 µl of 80% ethanol into Row F (wells F1 to F12.)
    f) Add 50 µl of Buffer BE into wells 1 through 12 in a separate elution strip.

> **CAUTION:** In order to minimize evaporation of ethanol, it is highly recommended to proceed with the purification on the KingFisher immediately after filling the plate.

18. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:
    - 520304_FFPE_Ultra_DNA_Duo_Protocol.bdz

> **CAUTION:** Do not load the plate before pressing "Play".

19. Press "Play" and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
20. Close the front lid while the KingFisher is running.
21. After the run is complete, a final prompt will appear: "Unload DNA Plate and DNA Elution Strip". Press the "Check Mark", unload the DNA Plate and cap the elution strip containing the DNA.

> **CAUTION:** It is normal for the final elution volume to be 10 to 15% less than the input due to loss during instrument run.

Short-term (1 to 2 days) storage of isolated DNA should be at 2 to 8 °C. For longer term, store the DNA at -20 °C.

Appendix: Troubleshooting Guide

Issue	Cause	Solution	Comments / Suggestions
Low yield of DNA	First proteinase K incubation too long.	Optimize the 1st proteinase K digestion step for your tissue samples.	During the 1st incubation step with Proteinase K at 56 °C, the RNA is released, and most of the DNA stays in the remaining tissue. If the PK digestion step is too long, the tissue will be over digested resulting in the release of the DNA into the solution.
	Parts or entire tissue pellet lost during supernatant removal.	Repeat using narrow mouth 200 µl pipette tip to take off RNA-containing supernatant.	Pipette from the top of the liquid surface and lower the tip as the volume decreases using a narrow pipette tip.
	Low tissue to wax ratio in FFPE section.	Trim off any excess paraffin before sectioning a FFPE tissue block. Repeat the procedure using additional sections until desired yield is achieved.	In your initial use of the truXTRAC FFPE total NA (tNA) Ultra Kit - Magnetic Bead, use FFPE blocks that have been well characterized for yield and quality.
	Insufficient tissue input.	Select FFPE section with higher tissue to wax ratio or add additional section.	See sample input guidelines in Section-1.
Low yield of RNA	Low tissue to wax ratio in FFPE section.	Trim off any excess paraffin before sectioning a FFPE tissue block. Repeat the procedure using additional sections until desired yield is achieved.	In your initial use of the truXTRAC FFPE total NA (tNA) Ultra Kit - Magnetic Bead use FFPE blocks that have been well characterized for yield and quality.
	Insufficient tissue input.	Select FFPE section with higher tissue to wax ratio or add additional section.	See sample input guidelines in Section-1.
Residual Beads seen in Bind and Wash Buffer wells after the KingFisher run	High wax in samples.	Lower input amount if possible. It is normal to see some residual beads in these wells due to beads trapped in paraffin	N/A
Eluates are cloudy	Residual paraffin in elution.	Spin the eluate for 30 seconds at 10,000 rcf. The residual wax will form a layer on top of the liquid and the aqueous solution can be transferred to a new tube.	If the paraffin emulsion was not completely removed in the wash steps, residual wax can be carried through to the elution step.

Tlips for Determining Quality and Quntity of the Purified DNA/RNA

- To determine DNA and RNA yields, a fluorometric assay such as Qubit TM (Life Technologies) should be used.
- In addition, spectrophotometric analysis determining the A260/280 and A260/230 ratios will determine if protein or peptide/salt contamination is present in the sample.
- qPCR can be used to assess the amplifiability of isolated DNA as well as the presence of inhibitors. Note that DNA from FFPE tissue itself can act as inhibitor at high input concentrations due to the extensive damage (nicks, depurination, etc.). Therefore, a dilution series over at least 5 orders of magnitude starting with undiluted material of the extracted DNA should always be done when assessing quality by qPCR. An example is shown in Dietrich et al. Figure 1 [3].
- Fragment size analysis of the RNA can be used to estimate the quality of the RNA by comparing the $\%DV_{200}$ [4, 5]
- RT-qPCR can be used to assess the amplifiability of the isolated RNA and correlated to the success of sequencing. [5]

Additional NOTES

1. See following link: www.covaris.com/resources/protocols/ for updates to this document.
2. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the tissue type, mass, and previous handling of FFPE samples.
3. Covered by US Patent 9,080,167
4. Other patents pending

References

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4. Matsubara, T. et al. (2020). DV200 Index for Assessing RNA Integrity in Next-Generation Sequencing. BioMed research international, 2020, 9349132. https://doi.org/10.1155/2020/9349132
5. truXTRAC FFPE RNA Extraction and Purification – Quality Metrics for Clinical Applications Powered by Adaptive Focused Acoustics (AFA) Covaris, 2019