truXTRAC® FFPE
total NA Kit -
KingFisher™ Duo Prime Purification (25)

Adaptive Focused Acoustics® (AFA) -based
sequential RNA and DNA extraction from FFPE tissues on the
KingFisher™ Duo Prime Purification System

PN 520246

Patents Granted and Pending

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INTENDED USE

The truXTRAC® FFPE total NA (Nucleic Acid) Kit – KingFisher (PN 520246) 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 Kit 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 FFPE slide sections up to 20 µm in total material thickness (from a $10mm^2$ area), scrolls/curls up to 20 µm in total material thickness, and one core up to 1.4 mm in diameter. For samples of larger input sizes, the truXTRAC® total NA Plus Kit (PN 520255) using magnetic bead-based purification may be used for extraction and purification of DNA and RNA from FFPE samples.

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 B) 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
010450	A	6/18	Kit Release of truXTRAC FFPE total NA Kit – KingFisher
010450	B	7/18	Combined the 010445 and 010450 for a complete KingFisher protocol
010450	C	8/19	Removed tissuePICK, sectionPICK and related accessories

KIT CONTENTS

- Tissue Lysis Buffer 6 ml
- Proteinase K (PK Solution) 1.25 ml
- Magnetic Bead Suspension 0.5 ml
- Buffer BB3 25 ml
- Buffer WB3 38 ml
- Buffer WB4 38 ml
- RNA Elution Buffer 3.5 ml
- Buffer BE 7.5 ml
- microTUBE-130 AFA Fiber Screw-Cap FFPE 25 tubes

SDS INFORMATION IS AVAILABLE AT http://covaris.com/resources/safety-data-sheets/

STORAGE

Upon kit arrival, store the Proteinase K solution and Magnetic Bead Suspension at 2-8C.

Store all other kit components at room temperature.

KINGFISHER BINDIT PROTOCOL FILES

- RNA Purification BindIT Protocol File: 520246_FFPE_RNA_Duo_Protocol.bdz
- RNA Purification with DNase Treatment BindIT Protocol File: 520246_FFPE_RNA_DNAse_Duo_Protocol.bdz
- DNA Purification BindIT Protocol File: 520246_FFPE_DNA_Duo_Protocol.bdz

For the most up to date protocols visit: http://covaris.com/resources/protocols/

Protocol	# of Samples	Length (hours)
520246_FFPE_RNA_Duo_Protocol.bdz	12	1:00
520246_FFPE_RNA_DNAse_Duo_Protocol.bdz	12	1:37*
520246_FFPE_DNA_Duo_Protocol.bdz	12	0:47

**This protocol contains a pause at 0:58 that requires the addition of reagents to the plate.*

CAUTION: The BindIT protocol files should not be altered in any manner. Covaris only supports the use of the BindIT protocol files contained in this document and will not support instances where protocol files have been altered.

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)
- microTUBE-130 Centrifuge and Heat Block Adapter (Covaris, PN 500406)
- Dry block heater with block to accommodate 2 ml tubes or temperature-controlled water bath able to accurately heat between 50-90C

Required Chemicals and Enzymes

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

Optional Enzymes

- DNase TURBO DNA-free kit (Thermo Fisher Scientific, PN AM1907)
- Optional 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)

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.

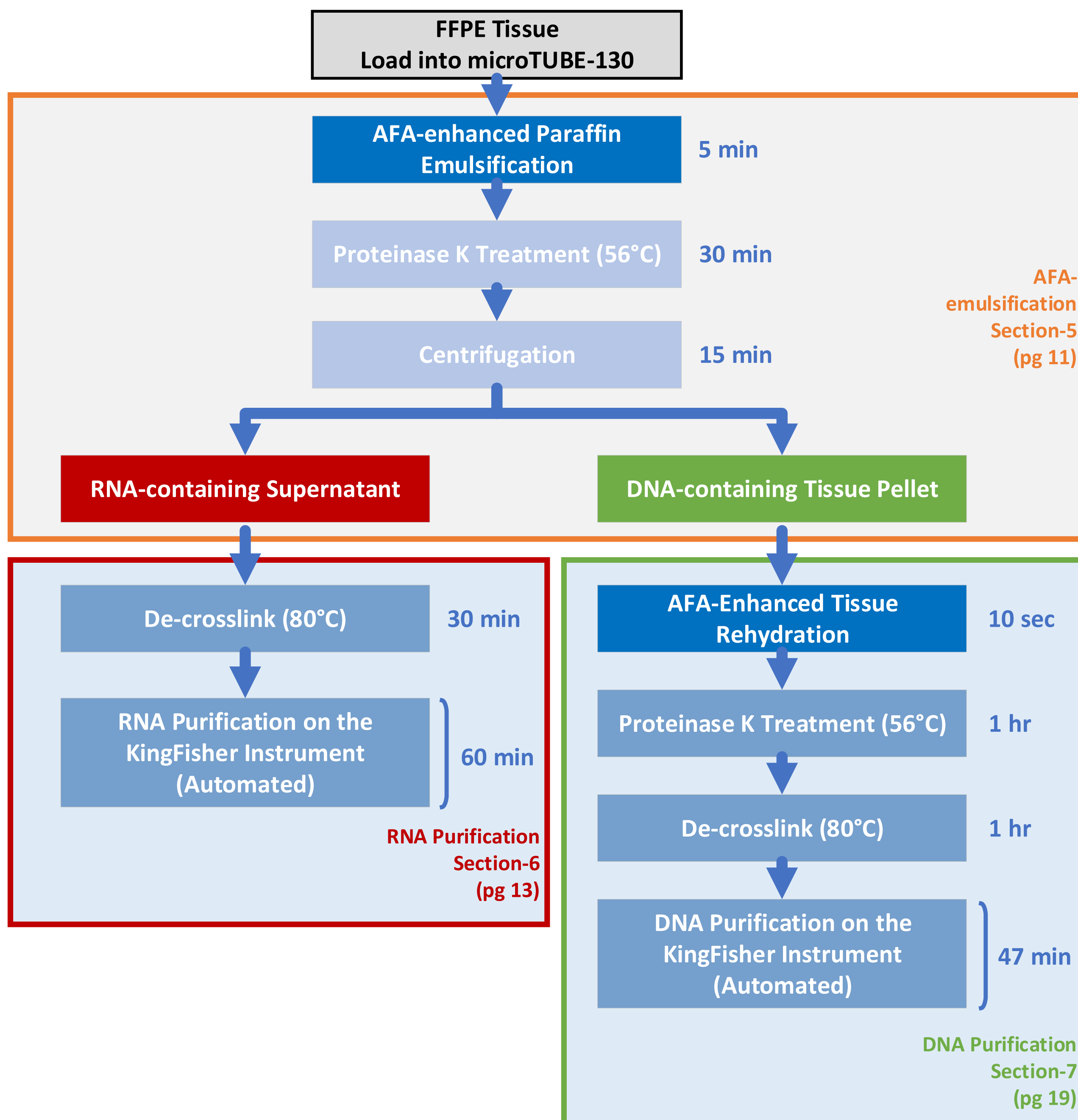
Instrument	M220	ME220	S220	E220 Evolution	E220	LE220 / LE220-plus
Holder/Rack Description (PN)	Holder XTU (500414)	Rack 4-place microTUBE Screw-Cap (500522)	Holder microTUBE Screw-Cap (500339)	Rack E220e 4 Place microTUBE Screw Cap (500432)	Rack 24 Place microTUBE Screw-Cap (500308)	Rack 24 Place microTUBE Screw-Cap (500308)
Plate definition file name	NA	<4 microTUBE-130 Screw-Cap PN 520216>	NA	<500432 Rack E220e 4 Place microTUBE Screw Cap>	<500308 Rack 24 Place microTUBE Screw-Cap>	<500308 Rack 24 Place microTUBE Screw-Cap>
Required Accessories (PN)	Insert XTU (500489)	ME220 Waveguide 4 Place (500534)	NA	Intensifier (500141)	Intensifier (500141)	NA

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 56C 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. For best results, minimize the amount of wax present by trimming. We recommend no more than 1 part wax to 1 part tissue.

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

Slides section input requirements:

Slide Collection Method	Maximum Input per microTUBE
Scalpel or razor blade to scrape material from slides	**20 µm of total thickness** **Area: 10 mm^2** **(2 slides at 5 µm thick = 10 µm total thickness)**

Curls/scrolls input requirements:

FFPE Curls/Scrolls Thickness	Maximum Scrolls per microTUBE
5 µm	**3**
10 µm	**2**
15-20 µm	**1**

Core punch input requirements:

FFPE Core Punch Outer Diameter	Maximum Core Punches per microTUBE-130
≤ 1.4mm (15 Gauge, outer) **Length = 5mm**	**1**

2 – Preparation of Reagents

Follow these instructions before starting the FFPE total NA isolation process.

- **80% ethanol:** Prepare 80% ethanol by mixing 4 parts 100% ethanol with 1 part nuclease free water. One sample requires 1.8 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 2 ml.

3 – Preparation of Heat Blocks

1. Preheat dry block heaters to 56C and 80C ± 3C. 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 microcentrifuge tube (1.5 or 2 ml) filled with water into the heat block.
    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 56C or 80C ± 3C.

CAUTION: The Covaris microTUBE must be used in conjunction with Covaris Centrifuge and Heat Block microTUBE Adapters (PN 500406).

It is important to use an accurate heating source for incubation of microTUBE-130s 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	M220	ME220	S220	E220 Evolution	E220	LE220
Peak Incident Power (PIP) (Watt)	75	75	175	175	175	450
Duty Factor (%)	20	25	10	10	10	30
Cycles Per Burst (CPB)	200	1000	200	200	200	200
Treatment time (seconds)	300	300	300	300	300	300
Bath temperature (°C)	20	20	18	18	18	18
Water Level (run)	Full	Auto	15	10	10	15

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	M220	ME220	S220	E220 Evolution	E220	LE220
Peak Incident Power (PIP) (Watt)	75	75	175	175	175	450
Duty Factor (%)	20	25	10	10	10	30
Cycles Per Burst (CPB)	200	1000	200	200	200	200
Treatment time (seconds)	10	10	10	10	10	10
Bath temperature (°C)	20	20	18	18	18	18
Water Level (run)	Full	Auto	15	10	10	15

5 – Paraffin Emulsification, Tissue Rehydration, & Lysis

1. Prepare Tissue 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 after preparation.

Table 1 – Tissue Lysis Buffer /Proteinase K Mix

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	**121** µl	**121** µl x N
Proteinase K Solution	**11** µl	**11** µl x N

* calculation includes 10% excess in final volume

2. Open the microTUBE-130 Screw-Cap and load the FFPE tissue.

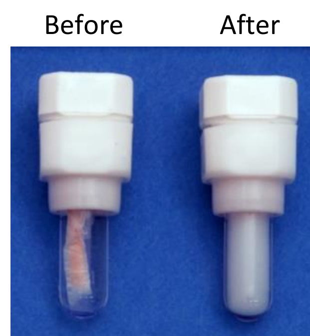
3. Add 120 µl Tissue Lysis Buffer/Proteinase K mix to the microTUBE-130.

4. Close the microTUBE-130 tightly with the Screw-Cap and transfer the microTUBE-130 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.

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

   Note: It is expected that the solution will turn milky white. See example below.

Before After



6. Remove the microTUBE-130 from the Focused-ultrasonicator and load it into the microTUBE-130 Centrifuge and Heat Block adapter.

   When processing in batches, samples may be kept at room temperature for up to two hours prior to Proteinase K incubation at 56C (Step 7).

7. Incubate for 30 minutes at 56C. Remove microTUBE-130 together with the microTUBE-130 adapter from the heat block, and let cool at room temperature for 3 min.

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

8. Place microTUBE-130 in the microTUBE-130 adapter with the bar code on the screw cap sleeve facing outward into a microcentrifuge (fixed angle rotor) and centrifuge at 5,000 x g for 15 minutes.

9. Open the microTUBE and carefully transfer 100 µl supernatant into a clean 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 on the same side as the barcode which 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 and barcode. DO NOT USE WIDE-MOUTH TIPS.
d. A layer of emulsified wax may descend obscuring the pellet. This is normal. Leave 10-20 µl of supernatant behind. This will not significantly impact RNA or DNA yield.

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

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

6 - RNA FFPE RNA PURIFICATION WITHOUT DNASE TREATMENT ON THE KINGFISHER

1. Set up the dry-heat blocks as explained in Section-3 and verify that the block temperatures have reached 80C (Step 2).

2. Incubate 2 ml microcentrifuge tube with the RNA-containing supernatant at 80C for 20 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 1.

3. After completing the decrosslinking, add the RNA containing lysate into row A of a 96 deep well plate.

4. Prepare BB3/Magnetic Bead Mix according to Table 2 below in a 5 ml tube.

Table 2 – BB3/Magnetic Bead Mix For RNA

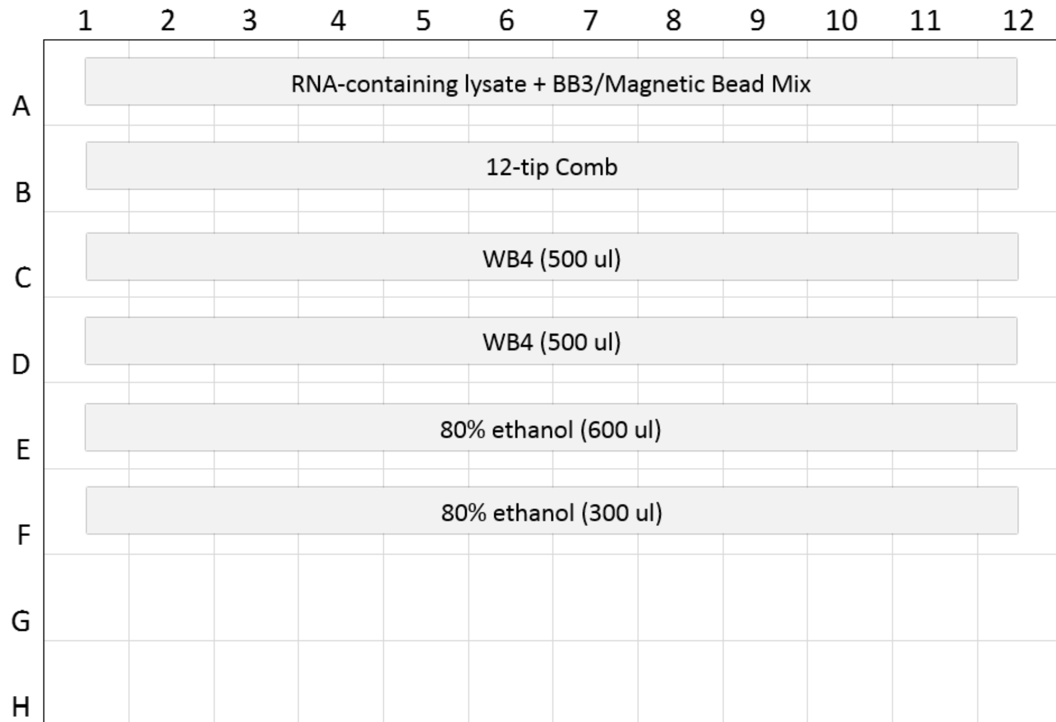
Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**330** µl	**330** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

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

5. Add 308 µl of BB3/Magnetic Bead Mix into Row A with the RNA containing lysate following the layout in Figure 1.

Figure 1: RNA Plate Layout



6. Set up the remainder of the RNA Plate as shown in Figure 1. (Use as many wells as needed per sample.)

a) Add the 12-tip comb to Row B.
b) Add 500 µl of WB4 into Row C (wells C1 to C12.)
c) Add 500 µl of WB4 into Row D (wells D1 to D12.)
d) Add 600 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 1-12 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.

7. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:

- 520246_FFPE_RNA_Duo_Protocol.bdz

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

8. Press “Play” and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
9. Close the front lid while the KingFisher is running.
10. 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 -80C.

7 - RNA FFPE RNA PURIFICATION WITH DNASE TREATMENT ON THE KINGFISHER

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

1. Set up the dry-heat blocks as explained in Section-3 and verify that the block temperatures have reached 80C (Step 2).

2. Incubate 2 ml microcentrifuge tube with the RNA-containing supernatant at 80C for 20 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. After completing the decrosslinking, add the RNA containing lysate into row A of a 96 deep well plate.

4. Prepare BB3/Magnetic Bead Mix according to Table 3 below in a 5 ml tube.

Table 3 – BB3/Magnetic Bead Mix For RNA

Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**330** µl	**330** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

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

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

Table 4 – DNase Mix

Reagent	Volume for 1 sample*	Volume for N samples*
Nuclease-free Water	**96.8** µl	**96.8** µl x N
10X DNase Buffer	**11** µl	**11** µl x N
TURBO DNase	**2.2** µl	**2.2** µl x N

* calculation includes 10% excess in final volume

6. Add 308 µl of BB3/Magnetic Bead Mix into Row A with the RNA containing lysate following the layout in Figure 2.

Figure 2: RNA with DNase Plate Layout

Row	Wells 1–12
A	RNA-containing lysate + BB3/Magnetic Bead Mix
B	12-tip Comb
C	WB4 (500 ul)
D	WB4 (500 ul)
E	80% ethanol (600 ul)
F	80% ethanol (300 ul)
G	
H	DNase Mix (100 ul)

7. Set up the remainder of the RNA Plate as shown in Figure 2. (Use as many wells as needed per number of samples.)

   a) Add the 12-tip comb to Row B.
   b) Add 500 µl of WB4 into Row C (wells C1 to C12.)
   c) Add 500 µl of WB4 into Row D (wells D1 to D12.)
   d) Add 600 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 1-12 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.

8. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:

- 520246 _FFPE_RNA_DNase_Duo_Protocol.bdz

CAUTION: Do not load the plate before pressing “Play”.

9. Press “Play” and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
10. Close the front lid while the KingFisher is running.
11. After 58 minutes, the instrument will prompt a message to remove the RNA Plate.
    a) Remove the RNA Plate from the KingFisher.
    b) Add 300 µl of BB3 into wells H1-H12.
    c) Place the RNA Plate back into the KingFisher.
    d) Press the “Check Mark”.
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 RNA at -80C.

8 – DNA PURIFICATION

1. Set up the dry-heat blocks as explained in Section-3 and verify the block temperatures to be 56C and 80C. The heat block set to 56C is required for Proteinase K incubation (Step 7). The heat block at 80C is required for DNA de-crosslinking (Step 9). Place the heat block adapters in the heat block set to 56C.

2. Prepare Tissue Lysis Buffer/Proteinase K Mix in a tube following the 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 minutes after preparation

Table 5 – Tissue Lysis Buffer/Proteinase K Mix for DNA

Reagent	Volume for one sample*	Volume for N samples*
Tissue Lysis Buffer	**88** µl	**88** µl x N
Proteinase K Solution	**22** µl	**22** µl x N

* calculation includes 10% excess in final volume

3. Open the microTUBE with the DNA-containing tissue pellet (Section-5 step 10) and add 100 µl of the Tissue Lysis Buffer/Proteinase K Mix for DNA. Re-cap the Screw-Cap microTUBE tightly.

4. Transfer the microTUBE-130 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.

5. Process the sample using the "***Acoustic Pellet Resuspension***" program on your Covaris Focused-ultrasonicator.

6. Remove the microTUBE-130 from the Focused-ultrasonicator and load the microTUBE into the pre-warmed microTUBE-130 Centrifuge and Heat Block adapter on the heat block set to 56C.

7. Incubate for 60 minutes at 56C.

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

CAUTION: It is recommended to increase the Proteinase K incubation time to 2 hours or up to overnight for >15 µm scrolls or 1.4 mm core sample inputs.

8. Remove microTUBE together with the microTUBE-130 adapter from the heat block and transfer directly to the dry heat block set-up for 80C incubation.

9. Incubate for 60 minutes at 80C.

10. Remove the microTUBE-130 from the heat block and let cool for 3 minutes at room temperature. Do not chill on ice.

A schematic plate map for DNA purification on the KingFisher Duo Prime Purification System is shown in Figure 3.

11. After completing the decrosslinking step, add the DNA containing lysate from the microTUBE-130 into row A of a 96 deep well plate.

12. Prepare BB3/Magnetic Bead Mix according to Table 6 below in a 5 ml tube.

Table 6 – BB3/Magnetic Bead Mix For DNA

Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**198** µl	**198** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

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

13. Add 188 µl of BB3/Magnetic Bead Mix into Row A with the DNA containing lysate following the layout in Figure 3.

Figure 3: DNA Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA-containing lysate + BB3/Magnetic Bead Mix											
B	12-tip Comb											
C	WB3 (400 ul)											
D	80% ethanol (600 ul)											
E	80% ethanol (300 ul)											
F												
G												
H												

14. 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 400 µl of WB3 into Row C (wells C1 to C12.)
    c) Add 600 µl of 80% ethanol into Row D (wells D1 to D12.)
    d) Add 300 µl of 80% ethanol into Row E (wells E1 to E12.)
    e) Add 50 µl of Buffer BE into wells 1-12 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.

15. Turn on the KingFisher Duo Prime and use the Directional Pad to select the following BindIT file:

- 520246_FFPE_DNA_Duo_Protocol.bdz

CAUTION: Do not load the plate before pressing “Play”.

16. Press “Play” and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
17. Close the front lid while the KingFisher is running.
18. 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 10-15% less than the input due to loss during instrument run.

Short-term (1 to 2 days) storage of isolated DNA should be at 2-8°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 56C, 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.	Follow guidelines in the protocol closely. Make sure laboratory personnel are trained in this procedure.
	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 kit, 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 kit 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 parrafin	
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.

TIPS FOR DETERMINING QUALITY AND QUANTITY OF THE PURIFIED FFPE DNA/RNA

- To determine DNA and RNA yields, a fluorometric assay such as Qubit ™ (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].

ADDITIONAL NOTES

1. See following link: http://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

1. Carrick et al. (2015) Robustness of Next Generation Sequencing on Older Formalin-Fixed Paraffin-Embedded Tissue. PLoS ONE 10(7): e0127353.
2. Landolt et al. (2016) RNA extraction for RNA sequencing of archival renal tissues. Scand J Clin Lab Invest 76(5):426-434.
3. Dietrich et al. (2013) Improved PCR Performance Using Template DNA from Formalin-Fixed and Paraffin-Embedded Tissues by Overcoming PCR Inhibition. PLOS one 8(10): e77771