KingFisher™ Duo Prime Purification in combination with the truXTRAC® FFPE total NA Plus Kit

Magnetic bead-based purification of RNA and DNA extracted from FFPE on the KingFisher™ Duo Prime Purification System

Addendum to Product PN 520255

INTENDED USE

The truXTRAC® FFPE total NA Plus Kit – Magnetic Bead (PN 520255) is intended for use in research applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

INTRODUCTION

This protocol is an Addendum to the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification (Product PN 520255). It describes the semi-automated magnetic bead-based purification of RNA and DNA on the KingFisher™ Duo Prime Purification System (Thermo Fisher Scientific, PN 5400110).

All reagents necessary for purification of FFPE total NA on the KingFisher™ Duo Prime are contained in the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead (PN 520255).

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 files is used during RNA and DNA purification from FFPE tissues.

Protocol	# of Samples	Length (hours)	Page
520255_FFPE_Plus_RNA_Duo_Protocol.bdz	12	1:26	3
520255_FFPE_Plus_RNA_DNAse_Duo_Protocol.bdz	12	2:08*	6
520255_FFPE_Plus_DNA_Duo_Protocol.bdz	12	1:20	9

**This protocol contains a pause at 1:27 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.

Note for first time users:

Please contact Covaris Application Support (ApplicationSupport@covaris.com) if you have any questions.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010457	A	6/18	Initial Release

PROTOCOLS AND KINGFISHER BINDIT PROTOCOL FILES

- truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification Manual
- RNA Purification BindIT Protocol File: 520255_FFPE_Plus_RNA_Duo_Protocol.bdz
- RNA Purification with DNase Treatment BindIT Protocol File: 520255_FFPE_Plus_RNA_DNAse_Duo_Protocol.bdz
- DNA Purification BindIT Protocol File: 520255_FFPE_Plus_DNA_Duo_Protocol.bdz

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

LABORATORY EQUIPMENT, CHEMICALS, AND CONSUMABLES TO BE SUPPLIED BY USER

Laboratory Equipment

- KingFisher™ Duo Prime Purification System (Thermo Fisher Scientific, PN 5400110)
- Dry block heater with block to accommodate 2 ml tubes or temperature-controlled water bath able to accurately heat between 50-60C

Chemicals

- Refer to the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification Manual

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)
- 2 ml nuclease free microfuge tubes (e.g., Eppendorf Safe-Lock Tubes, PN 022363352)
- Tube to make Binding Buffer/Bead Mixes (15 ml or 50 ml tubes)

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

1A - FFPE RNA PURIFICATION WITHOUT DNASE TREATMENT ON THE KINGFISHER

This protocol begins with the decrosslinked RNA containing supernatant (in a 2 ml microcentrifuge tube) that is obtained after **Step 2 in Section-6** in the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification Manual. A preheated dry block heater set to 56C is needed for Step 4.

CAUTION: Reagents must be prepared prior to starting this protocol as described in the truXTRAC® total NA Plus Kit – Magnetic Bead Purification Manual Section-2.

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

A schematic plate map for RNA purification on the KingFisher Duo Prime Purification System is shown in Figure 1.

1. Prepare BB3/Magnetic Bead Mix according to Table 1 below.

Table 1 – BB3/Magnetic Bead Mix For RNA

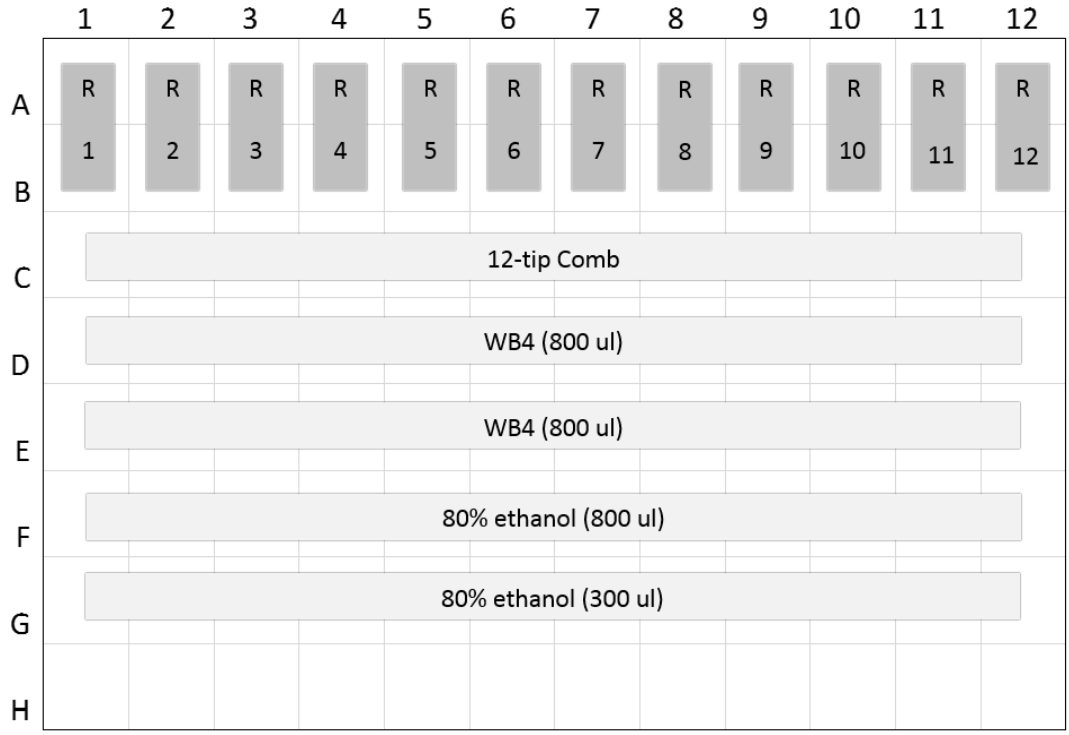
Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**1320** µl	**1320** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

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

2. Add 1208 µl of BB3/Magnetic Bead Mix to the RNA containing supernatant and cap the microcentrifuge tube.
3. Vortex the microcentrifuge tube for 5 seconds.
4. Incubate the microcentrifuge tube at 56C for 5 minutes.
5. Evenly distribute the mix into a single column in Rows A and B in a deep well 96 plate (~800 µl of the mixture into two wells), 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.

   a) Add the 12-tip comb into well row C.
   b) Add 800 µl of WB4 into wells D1 to D12.
   c) Add 800 µl of WB4 into wells E1 to E12.
   d) Add 800 µl of 80% ethanol into wells F1 to F12.
   e) Add 300 µl of 80% ethanol into wells G1 to G12.
   f) Add 50 µl of RNA Elution Buffer into wells 1-12 in an 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:

   - 520255_FFPE_Plus_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 long term storage, RNA should be kept at -80C.

1B - FFPE RNA PURIFICATION WITH DNASE TREATMENT ON THE KINGFISHER

This protocol starts with the decrosslinked RNA containing supernatant (in a 2 ml microcentrifuge tube) that was obtained after **Step 2 in Section-6** in the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification Manual. A preheated dry block heater set to 56C is needed for Step 4.

CAUTION: Reagents must be prepared prior to starting this protocol as described in the truXTRAC® total NA Plus Kit – Magnetic Bead Purification Manual Section-2.

The protocol allows you to purify RNA from 1-12 FFPE samples at a time with DNase treatment.

A schematic plate map for RNA purification on the KingFisher Duo Prime Purification System is shown in Figure 2.

1. Prepare BB3/Magnetic Bead Mix according to Table 2 below.

Table 2 – BB3/Magnetic Bead Mix For RNA

Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**1320** µl	**1320** 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.

2. Add 1208 µl of BB3/Magnetic Bead Mix to the RNA containing supernatant and cap the microcentrifuge tube.
3. Vortex the microcentrifuge tube for 5 seconds.
4. Incubate the microcentrifuge tube at 56C for 5 minutes.
5. Evenly distribute the mix into a single column in Rows A and B in a deep well 96 plate (~800 µl of the mixture into two wells), following the layout in Figure 2.

Figure 2: RNA with DNase Plate Layout

6. Prepare DNase Mix according to Table 3 below in a 2 ml tube. Mix gently by inversion.

Table 3 – 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

7. Set up the remainder of the RNA Plate as shown in Figure 2.

   a) Add the 12-tip comb into well row C.
   b) Add 800 µl of WB4 into wells D1 to D12.
   c) Add 800 µl of WB4 into wells E1 to E12.
   d) Add 800 µl of 80% ethanol into wells F1 to F12.
   e) Add 300 µl of 80% ethanol into wells G1 to G12.
   f) Add 100 µl of DNase mix into wells H1 to H12.
   g) Add 50 µl of RNA Elution Buffer into wells 1-12 in an 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:
    - 520255_FFPE_Plus_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 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 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 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 long term storage, RNA should be kept at -80C.

2 - FFPE DNA MAGNETIC BEAD PURIFICATION ON THE KINGFISHER

This protocol starts with the decrosslinked DNA containing lysate (in a 2 ml microcentrifuge tube) that was obtained after **Step 11 in Section-7** in the truXTRAC® FFPE total NA Plus Kit – Magnetic Bead Purification Manual. A preheated dry block heater set to 56C is needed for Step 4.

CAUTION: Reagents must be prepared prior to starting this protocol as described in the truXTRAC® total NA Plus Kit – Magnetic Bead Purification Manual Section-2.

The protocol allows for the purification of DNA from 1-12 FFPE samples at a time.

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

1. Prepare BB3/Magnetic Bead Mix according to Table 4 below.

Table 4 – BB3/Magnetic Bead Mix For DNA

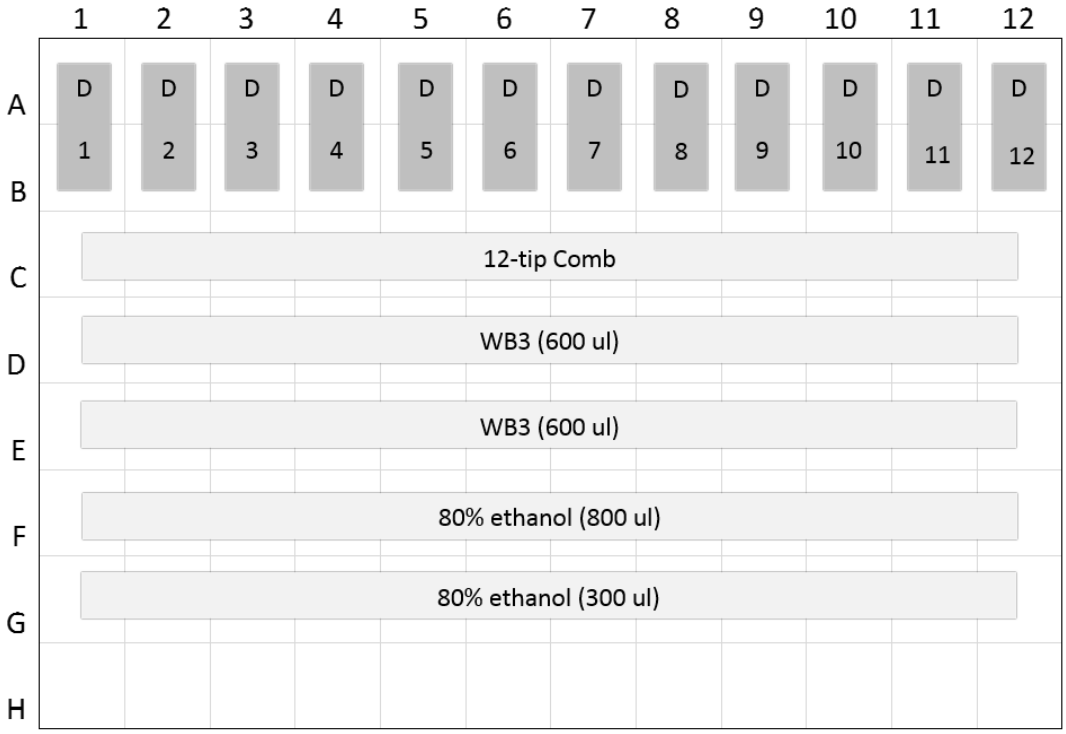
Reagent	Volume for 1 sample*	Volume for N samples*
BB3	**792** µl	**792** µl x N
Magnetic Bead Suspension	**8.8** µl	**8.8** µl x N

* calculation includes 10% excess in final volume

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

2. Add 728 µl of BB3/Magnetic Bead Mix to the DNA containing supernatant and cap the microcentrifuge tube.
3. Vortex the microcentrifuge tube for 5 seconds.
4. Incubate the microcentrifuge tube at 56C for 5 minutes.
5. Evenly distribute the mix into a single column in Rows A and B in a deep well 96 plate (~560 µl of the mixture into two wells), following the layout in Figure 3.

Figure 3: DNA Plate Layout



6. Set up the remainder of the DNA Plate as shown in Figure 3.

   a) Add the 12-tip comb into well row C.
   b) Add 600 µl of WB3 into wells D1 to D12.
   c) Add 600 µl of WB3 into wells E1 to E12.
   d) Add 800 µl of 80% ethanol into wells F1 to F12.
   e) Add 300 µl of 80% ethanol into wells G1 to G12.
   f) Add 50 µl of Buffer BE into wells 1-12 in an 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:

   - 520255_FFPE_Plus_DNA_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 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.