BECKMAN COULTER
Life Sciences

Total Nucleic Acid Isolation from Formalin-Fixed Paraffin-Embedded (FFPE) Tissues using FormaPure XL Total on a KingFisher™ Duo Prime

This method is applicable for scientists who want to extract DNA and RNA from FFPE using a semi-automated solution.

Please reference current FormaPure XL Total Instructions for Use for product information (C35992 or C35991)

Purpose

This protocol demonstrates the FormaPure XL Total performance on the KingFisher Duo Prime purification system. Automating chemistry can reduce the risk of human error, hands-on time (HOT) and total turn-around time (TAT).

Materials Used

Material	Part Number	Supplier
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AB02123	AmericanBio
KingFisher™ Duo 12 Tip Comb and 96 Deepwell Plates	97003530	ThermoFisher Scientific
Microcentrifuge tubes 1.5 mL	357448	Beckman Coulter Life Sciences
FormaPure XL Total	C35992 or C35991	Beckman Coulter Life Sciences
DNase (RNAase-free)	AM2222 or AM2224	ThermoFisher Scientific
Microcentrifuge	NA	NA
80°C Heat Block	NA	NA
60°C Heat Block	NA	NA

King Fisher Duo Prime Parameters

RNA Purification

RNA Purification Step	Plate Row	Reagent	Volume	Automation Parameters		
				Mixing Time/ Mixing Speed/ Pause Time	Collect Count/ time [s]	
DNase Treatment	A	DNAse I solution	100 µL	20 sec/ medium/ 20 min	5 / 30 sec	Heat 37°C for 20 min Pause and add 150 µL of RBA
Bind	B	BBA	150 µL	20 sec/ medium/ 5 min	5 / 30 sec	
		Lysate	100 µL			
Wash	C	80% Ethanol	375 µL	20 sec/ medium/ 5 min	5 / 30 sec	
Wash	D	80% Ethanol	375 µL	20 sec/ medium/ 5 min	5 / 30 sec	
NA	E	NA	NA	NA		
NA	F	NA				
NA	G	KingFisher™ Duo 12-Tip Comb				
Elute	H	Nuclease Free Water	40 µL	20 sec/ medium/ 5 min	5 / 30 sec	

DNA Purification

DNA Purification Step	Plate Row	Reagent	Volume	Automation Parameters	
				Mixing Time/Mixing Speed/Pause Time	Collect Count/ time [s]
Elute	A	Nuclease Free Water	40 µL	20 sec/ medium/ 5 min	5 / 30 sec
Bind	B	BBA	150 µL	5min / medium/ NA	5 / 30 sec
		Lysate	100 µL		
		RNase A	2.5 µL		
Wash	C	WBA	200 µL	20 sec/ medium/ NA	5 / 30 sec
Wash	D	80% Ethanol	375 µL20	20 sec/ medium/ NA	5 / 30 sec
NA	E	KingFisher™ Duo 12-Tip Comb	NA	NA	
NA	F	NA			
NA	G	NA			
Elute	H	NA			

Protocol

1. **Sample Preparation**
    a. Place 1-7 10 µM FFPE tissue sections in a 1.5 mL tube

2. **Deparaffinization**
    a. Add **450 µL** of **MO** to each tube and immerse the sections completely with a pipette tip
    b. Incubate the tube for **5 min** at **80°C**
    c. Vortex the sample twice for **5 seconds** each time to solubilize the paraffin and disperse the tissue

3. **Lysis**
    a. Add **200 µL** of **LBD** to the tube
    b. Centrifuge sample for **15 sec** at **10,000 g**
    c. **Incubate** the tube for **5 min** at **80°C**
    d. **Incubate** the tube for **5 min** at **room temperature**
    e. Add **30 µL** of **proteinase K**
    f. **Mix** by pipetting up and down 10 times in the bottom layer, or until thoroughly mixed.
    g. **Incubate** the tube for **2 hours** at **60°C**

4. **Automated RNA Processing**
    a. Transfer **100 µL** of **lysate** to KingFisher RNA Plate
    b. Load the KingFisher with the FormaPure XL Total RNA script
    c. Press **START** button
    d. Insert the RNA plate into the KingFisher per the instruments instructions
    e. During **PAUSE** (DNAse step) add **150 µL** of **RBA**
    f. After the KingFisher Duo Prime is complete, remove the plate
    g. Transfer and **Save** the supernatant in Row H to a plate for storage

5. **Automated DNA Procession**
    a. **Incubate** the tube for **1 hour** at **60°C**
    b. **Incubate** the tube for **1 hour** at **80°C**
    c. Transfer **100 µL** of **lysate** to KingFisher DNA Plate
    d. Load the KingFisher with the FormaPure XL Total DNA script
    e. Press **START** button
    f. After the KingFisher Duo Prime is complete, remove the plate
    g. Transfer and **Save** the supernatant in Row A to a plate for storage

Example Data

DNA and RNA were extracted from three 10 µM curls of breast, intestine and lung FFPE tissue. The average yields shown in Figure1 were calculated by Quant-iT assay (ThermoFisher Scientific). The yields for RNA and DNA are similar between manual and automation. The percent DV200 values for RNA and DNA extracted from the three tissues using FormaPure XL total manually and on the KingFisher™ Duo Prime are not significantly different from each other (T-test RNA p=0.8 and DNA p=0.3) the average percent DV200 values for RNA for each tissue type is in the table below.

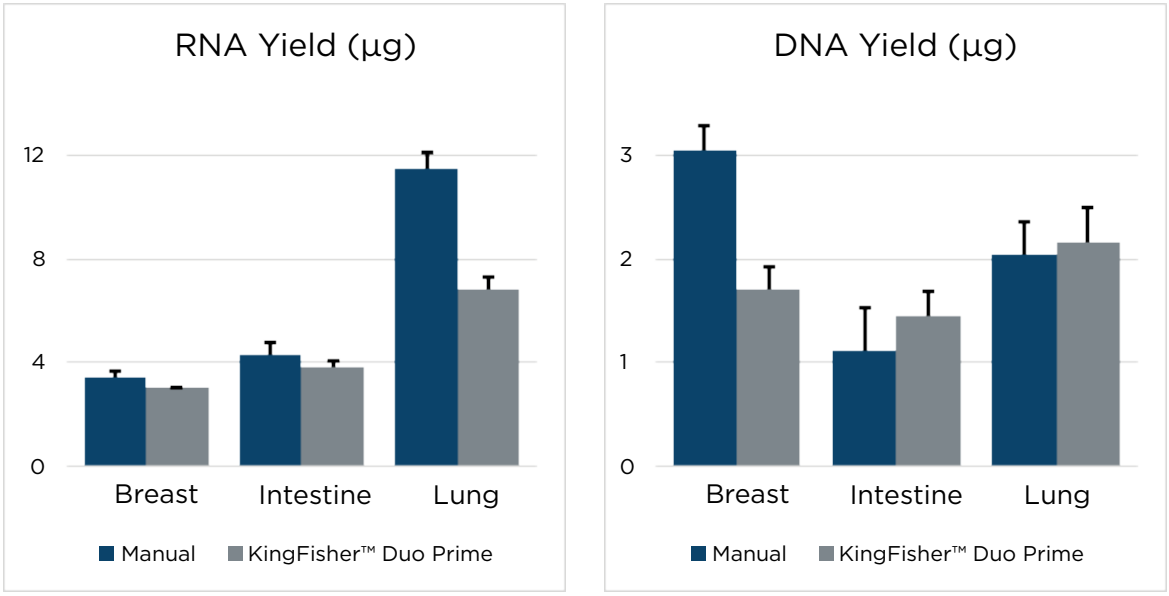

Figure 1. The average yield from three technical replicates of RNA and DNA extractions completed manually and on a KingFisher Duo Prime. The bars are an average yield of three technical replicates calculated by Quant-IT assay (Thermo Fisher Scientific). Error bars are the standard deviation of three technical replicates.

	RNA		DNA	
	Manual	**KingFisher Duo Prime**	**Manual**	**KingFisher Duo Prime**
Breast	84.7	88.5	88.8	86.3
Intestine	76.3	84.8	86.8	88.7
Lung	72.4	69.5	83.0	82.2

Table 1. The average percent DV200 values for RNA and DNA extracted from three tissue types. The percent DV200 values are not significantly different between the nucleic acid extracted manually or on the KingFisher Duo Prime.

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