Simultaneous, High-yield Extraction of DNA and RNA from FFPE Tissue

with the Ionic® FFPE Complete Purification Kit

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INTRODUCTION

Simultaneous extraction and purification of both RNA and DNA from FFPE tissue samples is critical to enable multiple downstream analyses from rare or limited samples. To avoid yield loss, most technologies use a serial isolation approach where DNA and RNA are separated after lysis by filtration or precipitation before entering separate purification workflows. This results in twice the number of purification steps per sample.

With simultaneous extraction and purification of RNA and DNA from FFPE tissue samples without yield loss, the Ionic® FFPE Complete Purification Kit for the Ionic® Purification System offers a significant savings on total workflow time and reduces hands-on time by more than 75%.

FIGURE 1: Ionic® Purification System

Isotachophoresis Technology

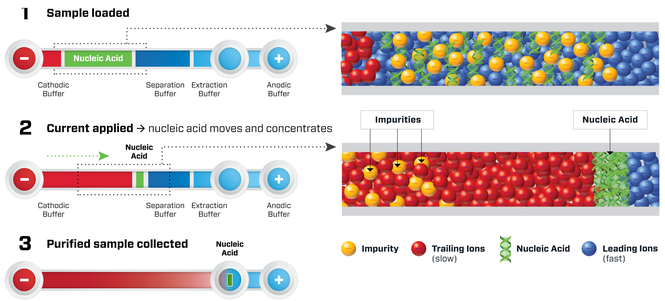

FIGURE 2: Purigen Isotachophoresis technology

The Ionic Purification System is a compact benchtop instrument that enables the automated purification of nucleic acids from a wide range of sample types including FFPE tissue samples and cultured or sorted cells. The Ionic System uses an innovative isotachophoresis technology to isolate the nucleic acids without binding to or stripping from physical surfaces. Biological samples are gently lysed and added directly to the chip placed on the Ionic system. To enable isotachophoresis, an electrical current is applied to the chip causing nucleic acids to separate in solution solely based on their charge and inherent electrophoretic mobility. As nucleic acids separate from impurities they concentrate and travel down a fluidic channel to an extraction well for collection. Since the nucleic acids are not denatured or dehydrated, nor are they bound and stripped from fixed surfaces, the process minimizes fragmentation and eliminates the risk of contamination from beads or wash buffers. The prepared nucleic acids are ready for analysis by downstream techniques such as next-generation sequencing (NGS) or qPCR.

Ionic® FFPE Complete Purification Kit

The Ionic® FFPE Complete Purification Kit is used with the Ionic system to enable the automated purification of DNA and RNA, including microRNA from FFPE tissue samples. The kit provides a protocol, Ionic® Fluidic Chips and reagents to enable the Ionic system to automate DNA and RNA purification using an innovative isotachophoresis technology. Samples are prepared for purification on the Ionic system using a simple lysis procedure that can be automated using a programmable thermomixer without any need for micro-dissection or de-paraffinization using harsh chemicals.

FIGURE 3: Ionic® FFPE Complete Purification Kit

SIMPLIFIED WORKFLOW

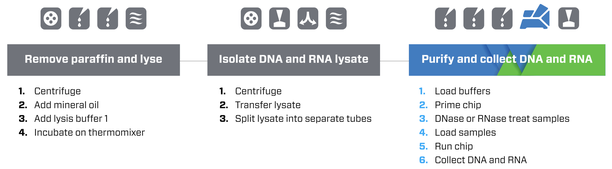

FIGURE 4: Description of the steps that occur across the stages of the Ionic FFPE Complete Purification Kit Workflow

Six adjacent sections of a 10 µm thickness were harvested from 6 FFPE tissue blocks containing brain, breast, colon, or lung tissue. DNA and RNA were extracted and purified from 4 of the 6 sections using the published workflow for the Ionic FFPE Complete Purification Kit (**FIGURE 4**). DNA and RNA were extracted and purified from the remaining sections using the published workflow for either a market-leading manual column-based kit or a market-leading manual bead-based kit.

	IONIC®	Manual Bead-based	Manual Column-based
Lysis time	**1.5** hrs	**Overnight**	**1** hr
RNA isolation	**2** hrs	**2** hrs	**2.5** hrs
Lysis time	**1.7** hrs	**3** hrs	**3.5** hrs
Total time	**5.2** hrs	**13** hrs	**6.5** hrs
Total hands-on time	**1.5** hrs	**6** hrs	**7** hrs

FIGURE 5: Comparison of hands-on time and total time to extract and purify RNA and DNA from 8 samples. Replicate 10 µm sections of FFPE samples were extracted and purified using either the Ionic system, a market-leading manual bead-based kit, or a manual column-based kit.

The average estimated time to process 8 samples through the Ionic FFPE Complete Purification kit was 5 hours and 12 minutes with a hands-on time of 1 hour and 30 minutes (**FIGURE 5**). This results in 11.25 minutes of hands-on time per sample to extract both DNA and RNA. The estimated time to process 8 samples through the column-based kit was 7 hours with most of that time being hands-on. This results in a hands-on time of 52.5 minutes per sample. Using a similar calculation, the hands-on time for the manual bead-based approach was 45 minutes per sample.

DATA

1.2x improvement to RNA Yield with Comparable DNA Yield

The simplified workflow of the Ionic FFPE Complete Purification Kit provides simultaneous extraction and purification of FFPE samples without compromising yield.

RNA Yield by Purification Method
10 µm sections

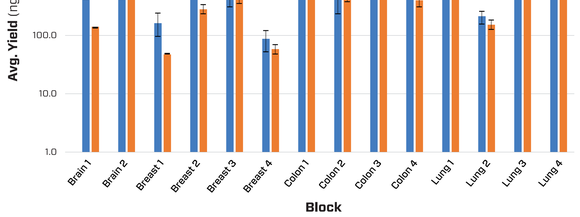

FIGURE 6: Replicate 10 µm sections from 14 FFPE tissue blocks were extracted and purified by both the Ionic FFPE Complete Purification Kit and a market-leading column-based DNA and RNA extraction kit. The extracted and purified material from each kit was measured using a Qubit fluorometer with the Qubit RNA High Sensitivity assay. In comparison to the column-based kit, the average yield improvement across the sample set for RNA purified using the Ionic system was 1.2x.

DNA Yield by Purification Method
10 µm sections

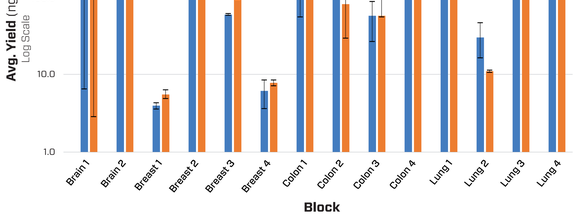

FIGURE 7: Replicate 10 µm sections from 14 FFPE tissue blocks were extracted and purified by both the Ionic FFPE Complete Purification Kit and a market-leading column-based DNA and RNA extraction kit. The extracted and purified material from each kit was measured using a Qubit fluorometer with the Qubit dsDNA High Sensitivity assay. The average yield performance of both methods is equivalent.

Improved DNA Yield with Optional Secondary Incubation

The Ionic FFPE Complete Purification Kit protocol includes an optional secondary incubation step to extend the lysis reaction of samples prior to DNA purification on the Ionic system. The extended incubation can increase the yield of DNA recovered from certain tissue types and greatly improves amplifiable yield.

With Overnight Incubation
by purification method, 10 µm sections

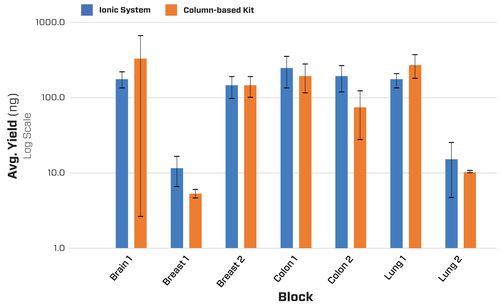

FIGURE 8: Replicate 10 µm sections from 7 FFPE tissue blocks were extracted and purified by both the Ionic FFPE Complete Purification Kit and a market-leading column-based DNA and RNA extraction kit. For the Ionic FFPE Complete Purification Kit, the lysate volume assigned for DNA extraction (50% of the total lysate) was incubated for an additional 7 hours prior to loading onto the Ionic system. The extracted and purified DNA from each kit was measured using a Qubit fluorometer with the Qubit dsDNA High Sensitivity assay. In comparison to the column-based kit, the average yield improvement across the sample set for DNA purified using the Ionic system was 1.2x.

With Overnight Incubation
by purification method, 10 µm sections

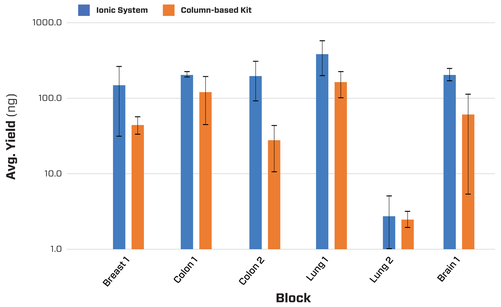

FIGURE 9: Replicate 10 µm sections from 6 FFPE tissue blocks were extracted and purified by both the Ionic FFPE Complete Purification Kit and a market-leading column-based DNA and RNA extraction kit. For the Ionic FFPE Complete Purification Kit, the lysate volume assigned for DNA extraction (50% of the total lysate) was incubated for an additional 7 hours prior to loading onto the Ionic system. The extracted and purified DNA from each kit was measured by qPCR using the Qiagen Multi-copy Reference Assay. In comparison to the column-based kit, the average yield improvement across the sample set for DNA purified using the Ionic system was 2.7x.

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