



PURIGEN BIOSYSTEMS

# Ionic® FFPE Complete Purification Kit

Simultaneous purification of DNA and RNA from FFPE samples



NUCLEIC ACID PURIFICATION PURE AND SIMPLE®

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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, the lonic<sup>®</sup> FFPE Complete Purification Kit for the lonic<sup>®</sup> Purification System offers significant savings on total workflow time and reduces total hands-on time by more than 75%.



FIGURE 1: Ionic® Purification System



#### **ISOTACHPHORESIS TECHNOLOGY**

FIGURE 2: Purigen isotachophoresis technology.

The lonic 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 lonic 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 lonic 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<sup>®</sup> 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<sup>®</sup> 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 deparaffinization using harsh chemicals.



FIGURE 3: Ionic® FFPE Complete Purification Kit

SIMPLIFIED 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 lonic 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.

The average estimated time to process 8 samples through the lonic 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.

## 1.2x improvement to RNA Yield with Comparable DNA Yield

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



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



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 columnbased 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 lonic 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 lonic 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 columnbased 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 lonic system. The extracted and purified DNA from each kit was measured by gPCR 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.

### 5



#### PURIGEN BIOSYSTEMS, INC.

SALES

5700 Stoneridge Drive, Suite 100 Pleasanton, CA 94588 USA

**TEL:** +1 877 PURIGEN (787-4436)

EMAIL: sales@purigenbio.com

#### SUPPORT

EMAIL: support@purigenbio.com TEL: +1 877 PURIGEN (787-4436)



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