IONIC® Purification System
Nucleic Acid Purification – Pure and Simple

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Why We Developed a New Approach to Nucleic Acid Purification

The commonly used bead- and column-based extraction technologies have followed the same fundamental workflow for over 20 years. This workflow uses ethanol, chaotropic salts, and other solutions to bind nucleic acid to a silica membrane or surface-labeled bead, which is then washed prior to the nucleic acid being stripped off the solid support into an elution buffer. During this typically laborious process, the nucleic acid is denatured, dehydrated, and fragmented. The eluate is also susceptible to contamination from wash buffers or beads.

### Disadvantages

<table>
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<tr>
<th>Incomplete binding to or removal from the solid support</th>
<th>Nucleic acid loss compromises data quality when sample input quantity is limited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery can be biased by fragment length or GC content</td>
</tr>
<tr>
<td></td>
<td>For researchers, reduced biological insight</td>
</tr>
<tr>
<td></td>
<td>For clinicians, less actionable information and false negatives</td>
</tr>
<tr>
<td>Contamination from wash buffers and bead coatings</td>
<td>Low purity leading to false negatives and compromised data</td>
</tr>
<tr>
<td>Workflow with multiple hands-on steps</td>
<td>Throughput bottleneck and potential errors; excessive use of disposable tips and labware</td>
</tr>
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</table>
Isotachophoresis, a Superior Approach to Nucleic Acid Separation

Isotachophoresis (ITP) separates and concentrates charged molecules in solution solely based on their electrophoretic mobility. Biological samples are gently lysed and added to the Purigen Ionic® Fluidic Chip. An electric field is then applied to the chip and the nucleic acid is isolated in its natural, native form. The nucleic acid is not denatured or dehydrated, and there’s no binding to, or stripping from, fixed surfaces. The result is a higher yield of pure nucleic acid that is less fragmented and free from bead or wash buffer contamination.

**Purigen Isotachophoresis**

**Separation Principle**

![Diagram of Isotachophoresis](image)

**Simple, Automated Charge-based Sample Prep in Solution**

1. **Sample loaded**
   - Sample loaded into Cathodic Buffer, Separation Buffer, Extraction Buffer, and Anodic Buffer.

2. **Current applied → nucleic acid moves and concentrates**
   - Current applied, nucleic acid moves and concentrates through the buffer layers.

3. **Purified sample collected**
   - Purified sample collected from Extraction Buffer and Anodic Buffer.

**Process and Characteristics**

- **Solution-based** → high yield and integrity
- **High purity**
- **Best representation of sample**
- **Result = better data**
Ionic® Purification System

How it Works

The Ionic® Fluidic Chip is placed on the Ionic system and separation buffers are loaded. The chip is then primed. Next, biological samples are added into the 8 sample wells and purification using isotachophoresis begins. By applying an electric field across the length of a chip microchannel, the Ionic system separates and concentrates nucleic acid between buffers with higher and lower mobilities. Impurities fall behind the low mobility buffer and are separated from target nucleic acids. As target nucleic acids pass through the channel, an integrated sensor stops the current once nucleic acids reach the extraction well.

The Next-Gen Sample Preparation System

The revolutionary Ionic Purification System requires no binding, stripping, or washing from fixed surfaces for higher yields, higher quality nucleic acids, and ultimately, better data for your research.

- No organic solvents
- No harsh, high-salt buffers
- No system programming
- No beads or repetitive washing
- No hands-on mixing, separation, sample transfers, or buffer exchanges
- No pumps, valves, or other moving parts
**Rapid Purification of Precious Samples**
in just ONE hour

**Ionic System Workflow**

1. **Load Run Buffers**
   - Add buffers from reagent kit – use touchscreen to start priming
   - **8 mins** (manual)

2. **Load Samples**
   - Load 8 samples – use touchscreen to start the run
   - **50 mins** (automated)

3. **Get Purified Nucleic Acid**
   - Collect samples when touchscreen displays “Run Complete”
   - **2 mins** (manual)

< 5 mins  
Total Hands-on Time per Sample  
60 mins  
Total Run-time

**Simplified Nucleic Acid Preparation**

The Ionic system is so different, its advantages are most readily understood in contrast with conventional nucleic acid extraction and purification methods:

- **Higher nucleic acid yields**  
  No sample loss associated with binding nucleic acids to, or stripping from, fixed surfaces

- **Simple workflows with fully automated separations**  
  No columns or beads and no repetitive washing

- **Reduced nucleic acid fragmentation**  
  No harsh high-salt buffers or organic solvents
Nucleic Acid that is Higher in Quantity and Quality

The Ionic system produces higher yields and higher quality nucleic acids due to less fragmentation and no risk of contamination from wash buffers or beads. When higher quality material is input into downstream analysis, superior results can be obtained; providing more biological insight for researchers or more robust and reliable results for clinicians.

1.5x – 2x More DNA from Low Cell Numbers

Consistently recover more DNA from as few as 10 cells

FIGURE 1: DNA extracted and purified from 3 cell lines at input amounts of 10, 100, 1000, 10,000 and 100,000 cells using the Ionic system and a commercially available column based kit. Ionic system yields increase linearly as input amount increases and range from 1.5x to 2x those of the column kit. Yield of DNA recovered on the Ionic system is 80-90% of theoretical maximum yield.

Less Fragmentation vs. Column-based Kits

gDNA Extracted from 56,000 Cells

FIGURE 2: 12 cell samples purified using Purigen and a conventional column-based approach (replicates of 8 for each technology). Samples purified with the ionic system show clean target bands; without smearing and less fragmentation.

Cell samples purified with the Ionic System demonstrate:

- Higher quality DNA with less fragmentation and no contamination
- Improved yield from as few as 10 cells
Simplified FFPE Workflow Saves Time and Money

Purigen’s FFPE protocols greatly simplify the processing of FFPE samples. For example, the Ionic® FFPE to Pure DNA protocol reduces the hands-on time to less than 3 minutes per sample and enables working directly from scrolls. The protocol also eliminates the need for a separate paraffin removal step.

**Column-based Kit Workflow**

1. **Sample**
2. **Remove Paraffin**
3. **Lyse**
4. **Heat**
5. **Binding**
6. **Wash Buffer 1**
7. **Wash Buffer 2**
8. **Spin Dry**
9. **Elute**
10. **Ready-to-use DNA**

*Compatible with scrolls or slides

Processed in batches of 24

< 3 minutes of hands-on time per sample

**Flexibility for Working with Scrolls or Slides**

The Ionic system produces more DNA and RNA from FFPE samples without requiring the use of slides (slide use and microdissection is optional). The ability to obtain comparable nucleic acid yields when using scrolls (versus slide mounted FFPE slices) greatly simplifies the workflow when sample micro-dissection is not required. This allows projects to be completed faster and at a lower cost.
Superior Nucleic Acid Recoveries from FFPE Samples

A vast majority of clinical samples used in oncology research are stored as FFPE tissues, which often contain degraded or fragmented nucleic acid. Conventional extraction methods are labor intensive and can further damage nucleic acid during the extraction and purification process. The Ionic system simplifies and accelerates nucleic acid purification, resulting in higher yields of higher quality DNA.

3.5x More DNA from FFPE Samples | (80bp target)

![Graph comparing nucleic acid yields from replicate sections of 32 FFPE samples purified by either the Ionic system or a commercially available column-based kit. The concentration of amplifiable DNA purified from each sample was determined with the Qiagen MRef Multicopy Reference Assay. For optimal performance from the columns, sections purified by this method were mounted onto slides prior to lysis. Sections purified by Ionic system were processed as unmounted scrolls to demonstrate improved performance using a simpler workflow. The Ionic system yield exceeds that of column-based extraction kit for 31 of 32 samples. Error bars indicate the 95% confidence interval for each data point.]

**Nucleic acid yields from scrolls using the Ionic Purification System are on average 3.5x higher when compared to yields from slide-mounted slices using a column-based kit.**

Higher Quality DNA vs. Column-based Kits

![Graph comparing nucleic acid quality using the QC Plex assay (Agilent) and resulting amplicons analyzed on the Agilent Technologies 4200 TapeStation. The resulting traces were scored using the DQC algorithm (Agilent). The results show better amplification from the samples purified with the Ionic system. In addition, 14 of the samples purified by column resulted in no amplification. Amplification was observed from every sample purified by the Ionic system.]

**FIGURE 3: Comparison of nucleic acid yields from replicate sections of 32 FFPE samples purified by either the Ionic system or a commercially available column-based kit.**

**FIGURE 4: Samples were assessed with the QC Plex assay (Agilent) and the resulting amplicons were analyzed on the Agilent Technologies 4200 TapeStation. The resulting traces were scored using the DQC algorithm (Agilent). The results show better amplification from the samples purified with the Ionic system. In addition, 14 of the samples purified by column resulted in no amplification. Amplification was observed from every sample purified by the Ionic system.**
Improved NGS Data Quality from FFPE Samples

In addition to increased yields and a greatly simplified workflow, data quality is also improved. Data below shows purified DNA from FFPE samples analyzed using the Agilent SureMASTR Tumor Hotspot sequencing panel which includes 252 amplicons ranging in size from 128–245 bps. To highlight coverage differences related to the sample purification technology, all data was normalized to a reference sample to remove the effect of coverage differences introduced by differences in target amplicon amplification efficiencies. The reference data set was generated using the average results obtained from the purification of a high-quality sample using both Ionic system and column-based techniques (as such the reference sample is not biased to either technology).

**More Uniform Coverage vs. Column-based Kits**

FIGURE 5: Results shown on a chromosome level. Ionic system eluates show tighter clustering more centered around the zero line. This is indicative of more uniform sequencing coverage.

**Less Coverage Bias vs. Column-based Kits**

FIGURE 8: Results shown relative to amplicon length and amplicon GC content. Ionic system samples show superior uniformity for both amplicon length and GC content, with very little deviation from the expected coverage. Column-based purification shows a bias towards shorter amplicon lengths and lower GC content amplicons.

- Ionic system purification shows no bias towards amplicon length.
- Ionic system purification shows no bias towards GC content.
A Better Solution for RNA from FFPE Samples

The Ionic Purification System provides for the automated purification of RNA from FFPE tissue samples with less hands-on time than conventional bead and column-based methods. To help scientists overcome the sample preparation bottleneck commonly associated with FFPE samples, the Ionic system provides a simple workflow that co-purifies both mRNA and miRNA with higher yields versus column-based extraction kits.

2x Higher Yields of RNA from FFPE Samples
(Qubit yield by purification method – 10 μm sections)

FIGURE 7: Replicate 10 μm sections from 17 FFPE tissue blocks were extracted and purified by both the Ionic FFPE to Pure RNA Kit and a market-leading column-based RNA extraction kit. The extracted and purified material from each kit was measured using a Qubit fluorometer and a Qubit High Sensitivity RNA assay. The average yield across the replicate samples processed by each method for each block is plotted as a bar graph.

Sample Summary

<table>
<thead>
<tr>
<th>Tissue</th>
<th>FFPE Blocks</th>
<th>FFPE Scrolls (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Column</td>
<td>IONIC®</td>
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<tr>
<td>BREAST</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>COLON</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>LIVER</td>
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<td>3</td>
</tr>
<tr>
<td>LUNG</td>
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<td>20</td>
</tr>
<tr>
<td>BRAIN</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Reference</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>65</td>
</tr>
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</table>

High Quality Sequencing Results

FIGURE 8: Libraries were prepared from RNA extracted and purified from replicate samples using the Ionic FFPE to Pure RNA Kit and a market-leading column-based RNA extraction kit following the AmpliSeq Illumina Immune Response Panel protocol. Libraries were then sequenced on an Illumina MiSeq sequencer. The mRNA expression results from each kit were normalized and compared by correlation analysis for each tissue type sampled. The correlation coefficient for each analysis indicates a strong correlation between the results of each kit for each tissue type sampled.
Get Higher miRNA Yields from FFPE Samples

For researchers studying either the relationship between gene expression and microRNA expression or focusing purely on microRNA expression in FFPE tissue samples, the Ionic system provides more miRNA than the market-leading column-based miRNA kit. More impressive, is that no additional steps are required. The Ionic FFPE to Pure RNA kit produces both mRNA and more miRNA from FFPE samples in a single, simple workflow.

**Total RNA Purification with Higher Yields of miRNA**

![Graph showing miRNA yield comparison between Ionic System and Column-based Kit](image)

**FIGURE 9:** RNA from replicate sections of 8 FFPE sample blocks were purified using either the Ionic FFPE to Pure RNA Kit or a column-based miRNA extraction kit. The extracted and purified samples from each kit were analyzed by qPCR and the Applied Biosystems TaqMan Advanced miRNA Assays for miR-16 and miR-21. The concentration of the target miRNA represented in each sample was extrapolated and plotted against the tissue type of the source FFPE sample block. The Ionic system produced samples with a higher concentration of miRNA in all but one of the samples tested. For several samples the column-based miRNA extraction kit did not yield a detectable amount of miRNA.

**Reproducible miRNA Expression Profiles**

![Graph showing miRNA expression correlation between Ionic System and Column-based Kit](image)

**FIGURE 10:** Samples from “Colon 1” of FIGURE 9 were analyzed for miRNA expression using the NanoString nCounter Human miRNA panel. The level of miRNA expression between replicate samples purified using the Ionic system has a Pearson correlation of 0.98. The level of miRNA expression between replicate samples purified using the Ionic system and the column-based miRNA kit has a Pearson correlation of 0.95. This analysis indicates a high reproducibility of miRNA expression across replicate samples purified using the Ionic system that is comparable to that of the column-based miRNA extraction kit.
Simultaneous Extraction of RNA and DNA from FFPE

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.

**Ionic® FFPE Complete Purification Workflow**

1. **Remove paraffin and lyse**
   1. Centrifuge
   2. Add mineral oil
   3. Add lysis buffer 1
   4. Incubate on thermomixer

2. **Isolate DNA and RNA lysate**
   1. Centrifuge
   2. Transfer lysate
   3. Split lysate into separate tubes
   4. Store DNA samples on ice

3. **Purify and collect DNA and RNA**
   1. Load buffers
   2. Prime chip
   3. DNase or RNase treat samples
   4. Load samples
   5. Run chip
   6. Collect DNA and RNA

**FIGURE 11**: Description of the steps that occur across the stages of the Ionic FFPE Complete Purification Kit Workflow

**Comparison of Total Hands-on Time vs. Manual Methods**

<table>
<thead>
<tr>
<th></th>
<th>Ionic</th>
<th>Manual Bead-based</th>
<th>Manual Column-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis time</td>
<td>1.5 hrs</td>
<td>Overnight</td>
<td>1 hr</td>
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<tr>
<td>RNA isolation</td>
<td>2 hrs</td>
<td>2 hrs</td>
<td>2.5 hrs</td>
</tr>
<tr>
<td>Lysis time</td>
<td>1.7 hrs</td>
<td>3 hrs</td>
<td>3.5 hrs</td>
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<tr>
<td>Total time</td>
<td>5.2 hrs</td>
<td>13 hrs</td>
<td>6.5 hrs</td>
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<tr>
<td><strong>Total hands-on time</strong></td>
<td><strong>1.5 hrs</strong></td>
<td><strong>6 hrs</strong></td>
<td><strong>7 hrs</strong></td>
</tr>
</tbody>
</table>

**TABLE 1**: In a study conducted by a third-party genomic services lab, this table shows 3 extraction methods that were used to compare the 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.

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 11). 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 Ionic FFPE Complete Purification kit was 5 hours and 12 minutes with a hands-on time of 1 hour and 30 minutes (TABLE 1). 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 Ionic FFPE Complete Purification Kit provides simultaneous extraction and purification of FFPE samples without compromising yield.

FIGURE 12: 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.

FIGURE 13: 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.

**Without Overnight Incubation**

by purification method, 10 μm sections

**With Overnight Incubation**

by purification method, 10 μm sections

**FIGURE 14:** 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. 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.

**FIGURE 15:** 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.

2.7x Increase in Yield with Overnight Incubation
# Purigen Products

## Instrument Configuration Part No.

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<thead>
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<th>Ionic® Purification System</th>
<th>Configuration</th>
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### Warranty Information

- 12 months coverage
- Initial response within 8 business hours
- On-site response within 3 business days
- Includes parts and materials
- On-site labor

## Kits Configuration Part No.

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