Streamlined DNA/RNA Co-Extraction from the Same FFPE Sample with Faster Turnaround Time

An updated method for sequential isolation of DNA and RNA into two fractions from the same FFPE sample with reduced upfront incubation times.

Objective

The objective of this technical note is to detail the updated and improved protocol of the Mag-Bind® FFPE DNA/RNA Kit and present the performance data compared to the original protocol. This improvement aims to significantly enhance laboratory efficiency and turnaround time, enabling higher throughput and quicker data delivery.

Kit

Mag-Bind® FFPE DNA/RNA Kit (M6955) Old Protocol vs Updated Protocol

Sample Types

Normal human FFPE liver

Sample Amounts

3 x 10 µm FFPE slices

Analysis

NanoDrop™ 2000c, RT-qPCR, qPCR, Infinium® FFPE QC Kit & TapeStation®

Kit Updates at a Glance

- Incubation times reduced by 3 hours to improve turnaround time.
- Optimized buffer volumes for maximizing DNA and RNA yield and quality.
- Expanded deparaffinization options for safety and convenience.
- Additional automation options add flexibility.

Automation Capabilities

- Scripts available for the Hamilton Microlab® STAR™, Dynamic Devices' Lynx®, MagBinder® Fit^{24}, and KingFisher™ magnetic particle processors.

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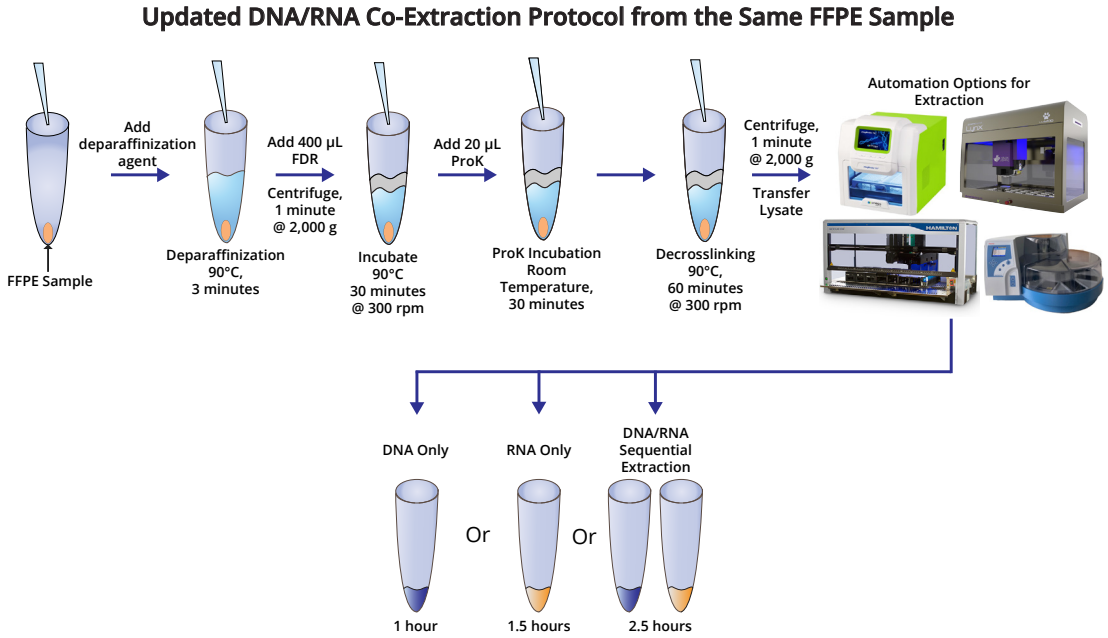

Figure 1. Illustration of the updated protocol for dual-fraction extraction of DNA and RNA from the same FFPE sample.

Methods

The Mag-Bind® FFPE DNA/RNA Kit was used to extract DNA and RNA in two fractions from 3x10 µm FFPE slices of normal human liver. Results were obtained from both the original extraction protocol, as well as the updated extraction protocol (**Figure 1**), and compared. The total time spent on pre-processing steps was reduced from ~5 hours with the original protocol to ~3 hours with the updated protocol (**Figure 2**). **Table 1** details the changes made to the kit and protocol in the update.

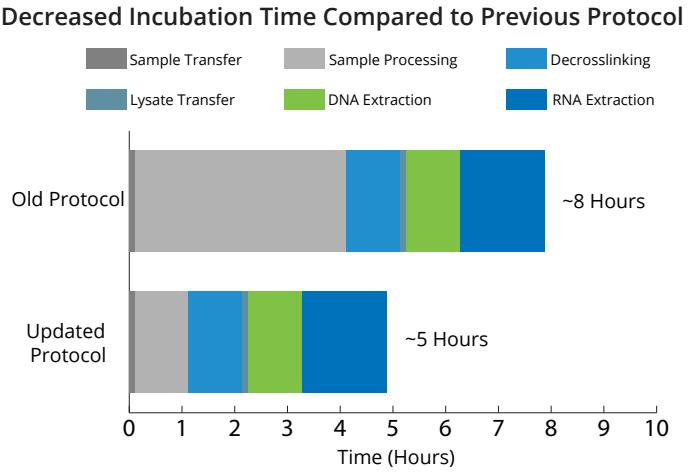

Figure 2. The updated protocol significantly reduces the total time required to extract both DNA and RNA from 7 hours and 53 minutes to 4 hours and 53 minutes by decreasing the upfront incubation time.

Table 1. Overview of updates made to the Mag-Bind® FFPE DNA/RNA Kit components and protocol. Protocol updates include variation in FDR and MB4 buffer volume addition, incubation times, temperatures, and shaking during various steps (refer to Product Manual).

Element	Updated M6955 Change
Kit Components	Unchanged
Buffer Volumes	More FDR and MB4 buffers are included M6955-00 • FDR Buffer increased from 35 mL to 50 mL • MB4 - unchanged M6955-01 • FDR Buffer increased from 140 to 180 mL • MB4 increased from 250 to 275 mL
Deparaffinization	Validated with other non-xylene solvent alternatives such as HistoChoice, HistoClear, HistoClear II, or hexadecane along with mineral oil.
Upfront Incubation	Significantly reduced from 4h at 56°C to 1h at 90°C.

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Results

Thermo Scientific's NanoDrop™ 2000c spectrophotometer was used to quantify the yields and purities of isolated DNA and RNA from both the old and updated extraction methods. DNA and RNA yields, as well as purities, were comparable between protocols (**Figure 3**).

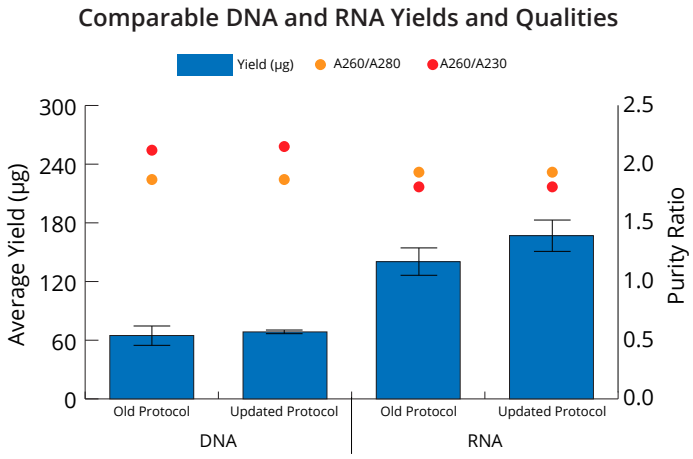

Figure 3. DNA and RNA were extracted from 3x10 µm FFPE slices of normal human liver using both the old and updated extraction protocol for the Mag-Bind® FFPE DNA/RNA Kit. The average yields and purities were comparable between extraction protocols.

DNA and RNA extracted using both the old and updated extraction protocol were analyzed via Agilent's TapeStation®. The original and updated protocols showed similar banding patterns for both DNA and RNA (**Figures 4 and 5**), indicating comparable DNA and RNA integrity between the old and updated protocols.

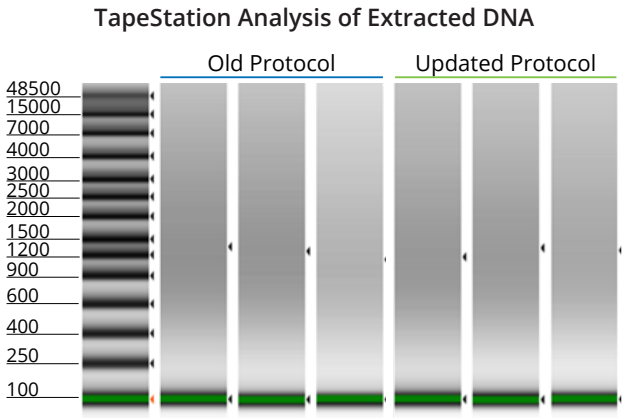

Figure 4. DNA and RNA were co-extracted from 3x10 µm FFPE slices of normal human liver using both the old and updated extraction protocols for the Mag-Bind® FFPE DNA/RNA Kit. The extracted DNA was analyzed using Agilent's TapeStation® 2200. Banding patterns were similar between the old and updated protocols.

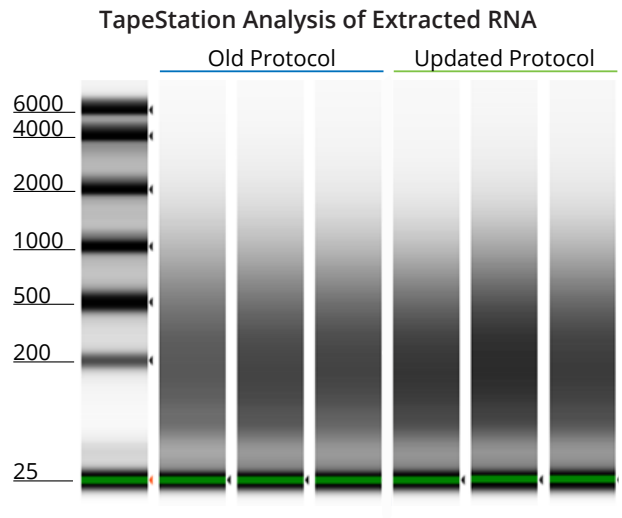

Figure 5. DNA and RNA were co-extracted from 3x10 µm FFPE slices of normal human liver using both the old and updated extraction protocols for the Mag-Bind® FFPE DNA/RNA Kit. The extracted RNA was analyzed using Agilent's TapeStation® 2200. Banding patterns were similar between the old and updated protocols.

qPCR and RT-qPCR were used to assess the suitability for downstream applications of the extracted DNA and RNA, respectively (**Figure 6**). The ΔCts between 10-fold and 100-fold dilutions were comparable between extraction protocols and indicate inhibitor-free nucleic acids.

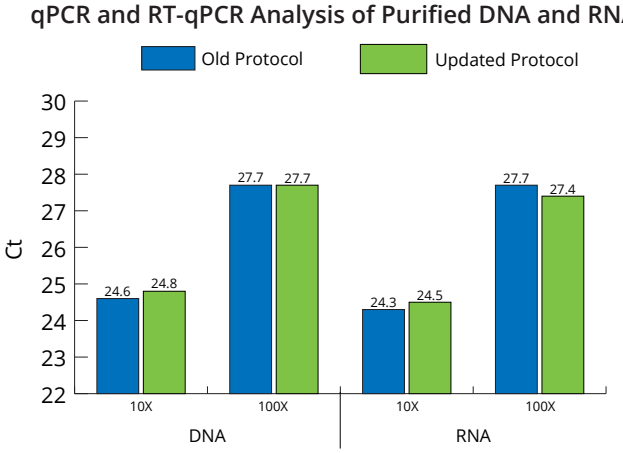

Figure 6. DNA and RNA were co-extracted from 3x10 µm FFPE slices of normal human liver using both the old and updated extraction protocols for the Mag-Bind® FFPE DNA/RNA Kit. ΔCts between 10-fold and 100-fold dilutions were comparable between extraction protocols for both DNA and RNA.

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The quality of DNA extracted using the old protocol vs the updated protocol was assessed using Illumina's Infinium® FFPE QC Kit and was found to be comparable (**Figure 7**). The ΔCt value compared to the QC standard was < 2 irrespective of the protocol used, which is indicative of the DNA's ability to be amplified and predicts assay success with sequencing platforms such as the TruSeq Amplicon Kit.

DNA Quality Assessment Using Illumina's QC Kit

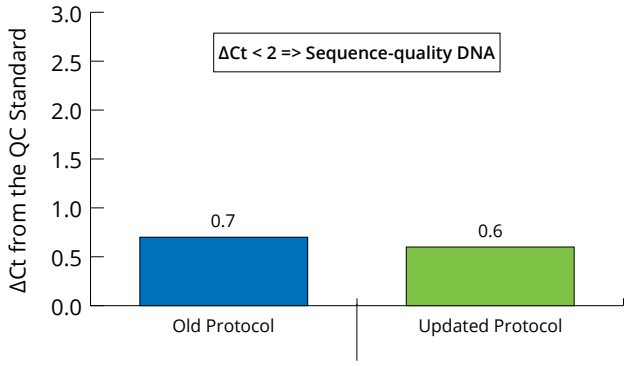

Figure 7. DNA was extracted from 3x10 µm normal human liver FFPE slices using both the old and updated extraction protocols for the Mag-Bind® FFPE DNA/RNA Kit. The quality of DNA extracted using both protocols was assessed with Illumina's Infinium® FFPE QC Kit. The results were comparable between protocols, and the ΔCt value compared to the QC standard was < 2 irrespective of the protocol used which implies sequence-quality DNA.

Conclusions

From the data presented here, it can be concluded that the updated protocol for the Mag-Bind® FFPE DNA/RNA Kit (M6955) is capable of co-extracting both DNA and RNA of comparable quality to the original protocol from the same FFPE sample. The updated protocol introduced a time savings of **3 hours** without sacrificing the yield or quality of the extracted nucleic acids, providing users with a streamlined solution for DNA and RNA extraction from their precious FFPE samples.

Product Information

Description	Product No.	Size
Mag-Bind® FFPE DNA/RNA Kit	M6955-00	1 x 96
	M6955-01	4 x 96

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