TECHNICAL NOTE

Maximizing DNA recovery from FFPE samples with the chemagic FFPE DNA Kit H96.

Abstract

Efficient extraction of high-quality DNA from formalin-fixed, paraffin-embedded (FFPE) samples remains a critical challenge in molecular pathology.

This study compares the performance of Revvity's chemagic™ FFPE DNA Kit H96 (CMG-1099) against a well-known competitor, revealing significant advantages in DNA yield, PCR amplification, and workflow efficiency. The results demonstrate the chemagic FFPE DNA Kit H96 as an optimal solution for unlocking the full potential of precious FFPE biospecimens.

Introduction

Formalin-fixed, paraffin-embedded (FFPE) preservation is a widely used technique for long-term archiving of clinical research specimens, including tissue and cytology samples. DNA and RNA purified from FFPE samples serve as valuable resources for pathologists and oncologists, allowing tumor mutation profiling to characterize cancers and guide the use of targeted therapeutics.

It is also being used by pharmaceutical and research labs for biomarker discovery and translational research. However, extracting DNA/RNA from these samples can be challenging as formaldehyde can cross-link fragments and chemically modify nucleic acids, impacting their downstream analysis.



The Revvity chemagic FFPE DNA Kit H96 (CMG-1099) addresses these challenges through an optimized procedure with a heated de-crosslinking step, which improves nucleic acid integrity and downstream assay performance. The kit is designed for total nucleic acid purification, with primary optimization for DNA extraction; co-purified RNA can also be utilized for downstream applications. Leveraging chemagic M-PVA magnetic bead technology, the kit enables high-throughput, automated processing with consistent yields, reducing hands-on time and minimizing operator variability.

The purpose of this study was to evaluate the performance of the chemagic FFPE DNA Kit H96 in comparison with a manual competitor FFPE DNA extraction kit, assessing DNA yield and PCR amplification efficiency across two sample types.

Materials and methods

Sample material

- Mimix™ BRAF Wild Type 100%, FFPE Reference Standard - 15 µm sections: Revvity: cat. no. HD266.
- Murine liver FFPE samples, 10 µm sections cut from the same block: University Clinique Aachen, Aachen, Germany.

DNA extraction

The manual extraction procedure was performed using the competitor FFPE DNA extraction kit according to manufacturer's instructions. Automatic extraction procedure was performed with the chemagic FFPE DNA Kit H96 (CMG-1099) on the chemagic 360 instrument according to the user guide. DNA was eluted in 100 µL of the corresponding elution buffers (figure 1).

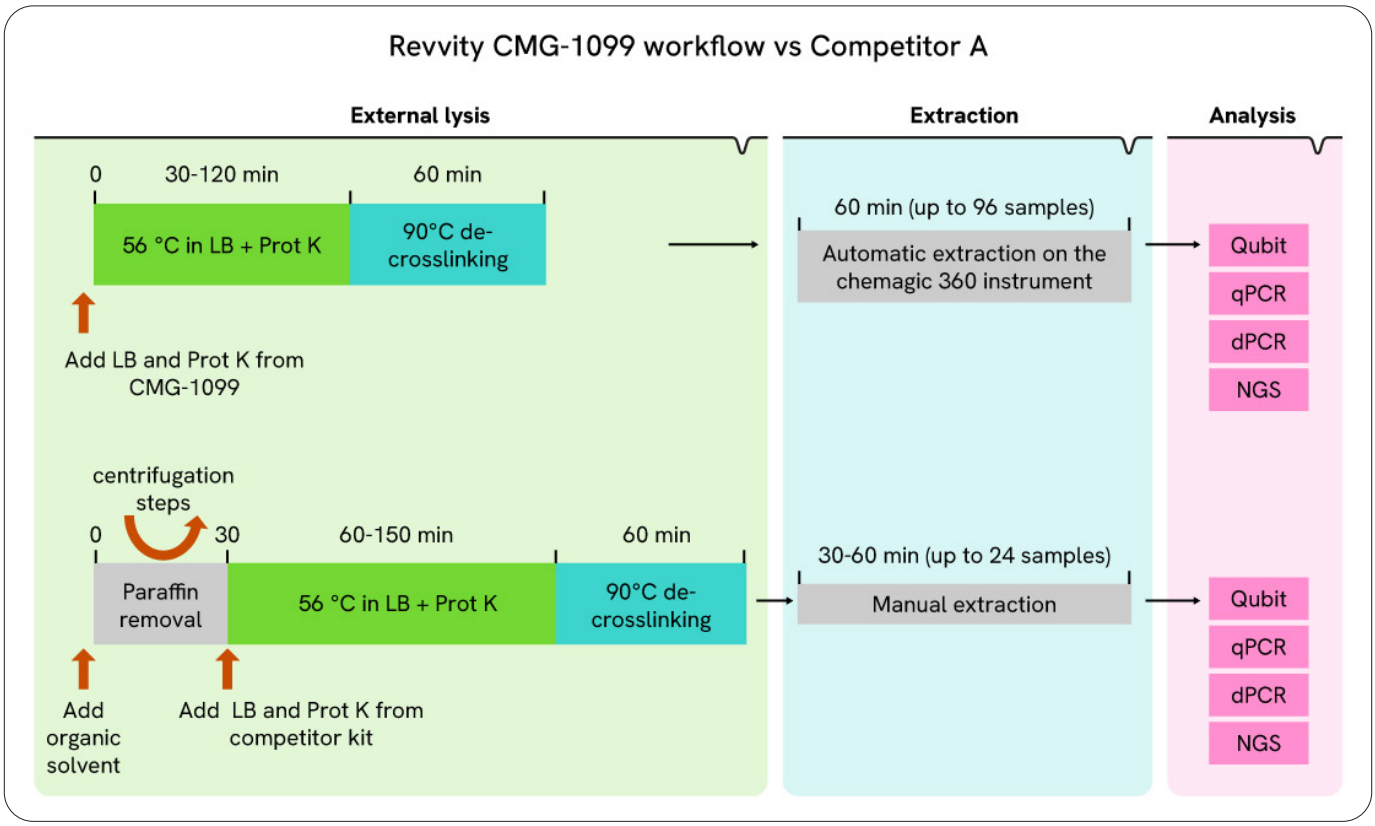


Figure 1: Revvity chemagic FFPE DNA Kit H96 workflow (above) in comparison with the competitor FFPE DNA extraction kit (below). The Revvity workflow avoids organic solvent handling and centrifugation steps and has a ~60 min shorter pre-treatment procedure compared to the manual workflow. Number of extractions can also be scaled up to 96 samples per run on the chemagic 360 instrument Lysis buffer (LB) and Proteinase K were used from each respective kit.

Concentration measurement and qPCR

DNA concentration was measured using Qubit™ DNA High Sensitivity or Qubit Broad Range kits (Thermo Fisher Scientific). Yields were calculated based on the actual eluate volume. Eluates were analyzed with qPCR targeting either human Alu repeats (for Mimix FFPE Reference Standards, Revvity Discovery) or murine *KRAS* gene (for murine liver FFPE samples) on a Quantstudio™ 5 platform (Thermo Fisher Scientific) with FastStart™ Universal SYBR™ Green Master Mix reagents (ROX) (Roche Diagnostics). Primers (see table 1) were used at a final concentration of 0.25 µM.

Table 1: qPCR primer sequences.

Name	Organism	Sequence	Annealing temp. (°C)	Fragment length
KRAS_fw	Mouse	5´-ttccctgggttttggactta-3´	60	146 nt
KRAS_rv		5´-gcttattatcctgcttcc-3´		
ALU_fw	Human	5´-cctgaggtcaggagttcgag-3´	64	115 nt
ALU-rv		5´-cccgagtagctgggattaca-3´		

Results

DNA was extracted from murine liver sections or Mimix cell line-derived FFPE Reference Standards using the chemagic FFPE DNA Kit H96 (CMG-1099) or a competitor FFPE DNA extraction kit.

DNA yields from extractions were measured and the corresponding qPCR performed. Comparative extraction performances of both kits are depicted on Fig.2 and Fig.3.

As shown in Figure 2A, the chemagic FFPE DNA workflow yielded significantly higher amounts of DNA compared to the competitor kit. Correspondingly, the qPCR targeting the murine KRAS gene showed lower C_T values for the samples extracted by the chemagic kit workflow, indicating more efficient amplification of the target sequence (Figure 2B).

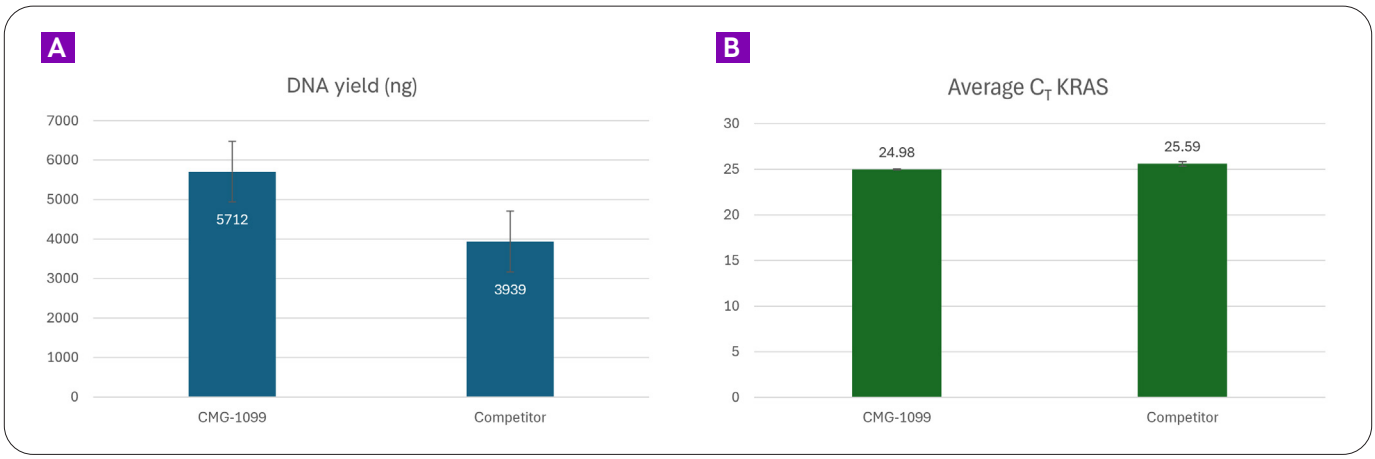


Figure 2: Comparative analysis of FFPE kits performance on murine liver FFPE samples. A. Average DNA yield was 5712 ng for the chemagic extraction workflow and 3939 ng for the manual competitor kit n=3; B. Mean CT values for KRAS qPCR. I indicate a slightly lower C_T value for the chemagic FFPE DNA kit workflow (average CT = 24.98) compared with the competitor kit (average CT = 25.59) n=3.

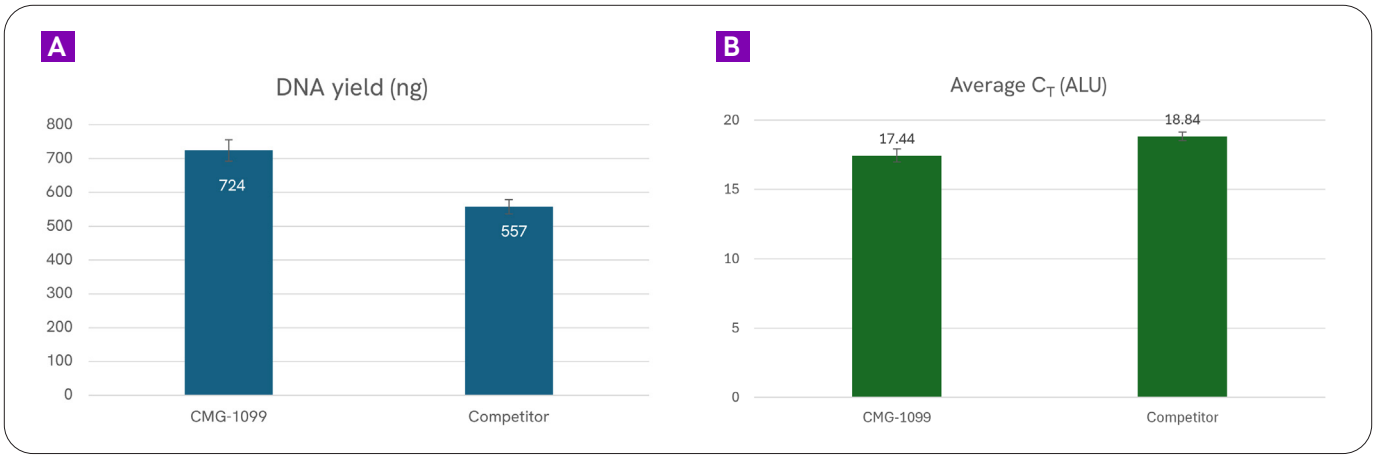


Figure 3: Comparative analysis of FFPE kits performance on Mimix cell line-derived FFPE standards. A. Average DNA yield for the chemagic FFPE DNA kit workflow was 724 ng and for the manual competitor kit 557 ng, n=3; B. The average C_T values for ALU qPCR were lower for the chemagic workflow (average C_T = 17.44) compared with the competitor kit (average C_T = 18.84) n=3.

A similar trend was observed with the human FFPE reference standards (Figure 3). The chemagic workflow resulted in higher DNA yields. Likewise, the human ALU repeat qPCR demonstrated lower C_T values for the samples extracted by the chemagic FFPE DNA workflow, reflecting the increased yield.

Overall, the chemagic FFPE DNA Kit H96 outperformed the competitor FFPE DNA extraction kit across both sample types, yielding higher quantities of DNA resulting in efficient downstream detection by PCR.

Summary

This study compared the DNA extraction performance of the chemagic FFPE DNA extraction kit (CMG-1099) on the chemagic 360 instrument with a commonly used manual competitor kit using both murine liver FFPE sections and human FFPE reference standards. The chemagic FFPE DNA Kit H96 consistently produced higher DNA yields across both sample types and lower C_T values in qPCR assays targeting KRAS (murine) and ALU repeats (human).

Additionally, the chemagic workflow offers an organic-solvent-free, centrifugation-free external lysis protocol, reducing hands-on time and exposure to hazardous chemicals while enabling high-throughput automation on the chemagic 360 instrument for up to 96 samples per run.

Scaling to lower throughputs or high-throughput fully automated workflows are also possible on the chemagic Prepito and the chemagic Prime family of instruments respectively as chemagic separation technology and M-PVA Magnetic Bead extraction chemistry are similar across all Revvity chemagic instruments.

The automated bulk dispensing of reagents saves hands-on time and reduces running costs in comparison with cartridges, making it possible for labs who perform routine pathology workflows to scale up when needed.

The gentle resuspension of magnetic beads with rotating chemagic rods also help preserve nucleic acid integrity[1] and studies have found it to be compatible with downstream DNA/RNA sequencing or NGS-based methylation assays[2,3,4,5].

This indicates the reliable quality of extracted DNA/RNA from FFPE samples using chemagic technology and applicability to even challenging assays such as genome-wide methylation arrays.

In conclusion, the chemagic FFPE DNA Kit H96 delivers high DNA extraction performance with improved workflow efficiency, making it an optimal choice for FFPE-based molecular research applications.

References

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