

Removal of Genomic DNA Contamination from Purified cfDNA Using Mag-Bind® TotalPure NGS: A cfDNA Enrichment Methodology

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Introduction

Cell-free DNA (cfDNA) has emerged as a tool of great potential in molecular diagnostics; however, cfDNA has not reached its peak utilization due to several hurdles obstructing high-quality extraction, such as the low concentrations in which cfDNA is found, as well as the high concentrations of contaminating genomic DNA (gDNA). Because of this, reliable methods for removing gDNA from cfDNA extractions are an interesting avenue for ensuring high-quality extractions. High quality cfDNA with minimal contamination is critical in improving the sensitivity and accuracy of downstream clinical diagnosis.

Blood was collected from five subjects and stored using Mawi HemaSure-OMICs tubes. cfDNA was extracted using the pre-filled MB Fit24™ cfDNA Kit (B3298-10-48PF) automated on the MagBINDER® Fit²⁴ Nucleic Acid Purification System. Following purification, high molecular weight gDNA contamination was removed using Mag-Bind® TotalPure NGS (M1378). Here, we compare the quality of extracted cfDNA before and after gDNA contamination has been removed using Mag-Bind® TotalPure NGS.

Materials and Methods

Sample Collection and Storage

Blood collected in Mawi HemaSure-OMICs tubes was left to sit at room temperature for 24 hours.

Extraction Kit

Omega Bio-tek's MB Fit24™ cfDNA Kit (B3298-10-48PF) was used for extraction of cfDNA from 4 mL working volume. Briefly, 2 mL plasma was combined with 2 mL elution buffer included with the kit to bring the starting sample volume to 4 mL. Lysis was carried out offline by incubating at 60°C for 30 minutes after addition of 60 µL ProK and 270 µL DS Buffer. The MB Fit24™ cfDNA Kit's cartridges come pre-filled with reagents in volumes and positions as shown in **Figure 1**.

Extraction Methodology

After lysis, the pre-filled reagent cartridges were loaded on the MagBINDER® Fit²⁴, and the pre-loaded extraction script for the MB Fit24™ cfDNA Kit was selected and ran on the instrument. All samples were run on the MagBINDER® Fit²⁴ instrument simultaneously with the magnetic rods working in unison to pick-up, transfer, and release magnetic particles in different wells of the reagent cartridge to provide purified cfDNA in the elution tube at the end. cfDNA was eluted in 100 µL volume, and the protocol time using the MagBINDER® Fit²⁴ was ~55 minutes from cartridge placement to cfDNA elution.

Yield and Quality

The yield and quality of the cfDNA was evaluated using the Cell-Free DNA ScreenTape Assay on the Agilent 4150 TapeStation® system, and its fraction relative to the total DNA extracted was assessed immediately following purification.

gDNA Removal

Genomic DNA removal with Mag-Bind® TotalPure NGS was tested on eluted cfDNA containing high molecular weight DNA contamination. These samples were subjected to double-sided size selection at 1.8X/0.55X ratio to selectively exclude higher molecular weight genomic DNA. After gDNA removal with Mag-Bind® TotalPure NGS, the cfDNA samples were again evaluated using the Cell-Free DNA ScreenTape Assay on the Agilent 4150 TapeStation® system and its fraction relative to the total DNA extracted was assessed and compared to cfDNA samples that were not subjected to cleanup with TotalPure.

Reagents Used in MB Fit24™ cfDNA Protocol

Position	Content	Volume
1	JSB Buffer	4 mL
2	GT7 Buffer v1.1	1 mL
3	GT7 Buffer v1.1	1 mL
4	eSPW Buffer	1 mL
5	eSPW Buffer	1 mL
6 (User Filled)	Elution Buffer	50 µL - 100 µL

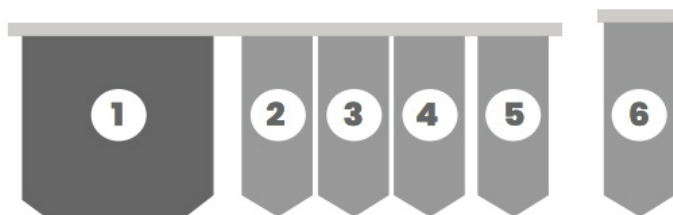


Figure 1. MB Fit24™ cfDNA Kit reagent cartridge positions, contents, and volumes.

Results and Discussion

TapeStation analysis using the Cell-Free DNA ScreenTape Assay was performed to evaluate the yield and quality of extracted cfDNA. The extracted cfDNA samples showed a great deal of genomic DNA contamination, with the cfDNA fraction ranging from 21-37% of the total DNA extraction across all the samples (see “No Cleanup” lanes, **Figure 2**).

Because the resulting extractions showed the large amounts of high molecular weight DNA, the purified cfDNA was subjected to genomic DNA cleanup using Mag-Bind® TotalPure NGS beads. It can be seen in **Figure 2** that the %cfDNA exhibited significantly increased from 21-37% to 94-97% after cleanup, substantiating that Mag-Bind® TotalPure NGS can remove high molecular weight DNA contamination from cfDNA extractions.

Conclusions

cfDNA is a powerful tool that is best exploited when minimal contaminating gDNA is present after extraction. The semi-automated workflow for extraction of cfDNA using the MB Fit24™ cfDNA Kit (B3298-10-48PF) on the MagBINDER® Fit²⁴ Nucleic Acid Purification System produces high-quality cfDNA suitable for use in downstream applications like qPCR. In addition, Mag-Bind® TotalPure NGS is capable of removing high molecular weight gDNA contamination from cfDNA extractions.

Product Information

Product	Description
Mag-Bind® TotalPure NGS	M1378-00, 5mL
	M1378-01, 50 mL
	M1378-02, 500 mL
MB Fit24™ cfDNA Kit	B3298-10-48PF
MB Fit24™ cfDNA Kit CE IVD	B3298-10-48PFCEIVD
MagBINDER® Fit ²⁴	B1-001-24
MagBINDER® Fit ²⁴ CE IVD	B1-001-24CEIVD

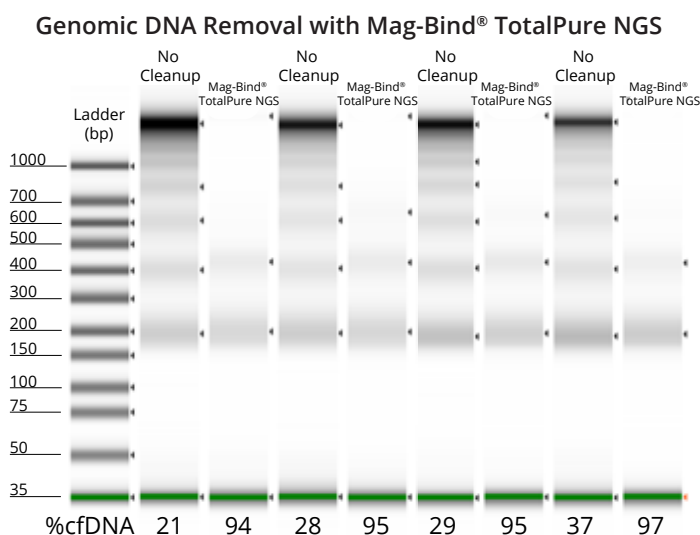


Figure 2. TapeStation analysis of genomic DNA cleanup using Mag-Bind® TotalPure NGS with cfDNA samples extracted from plasma stored in Mawi collection tubes.