Automated cfDNA Purification from up to Ninety-six, 4 mL Plasma Samples Using the Mag-Bind® NAP STAR

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Introduction

Cell-free DNA (cfDNA) has emerged as a revolutionary tool in the realm of molecular diagnostics, offering a non-invasive window into the genetic information of various bodily tissues. The development of cfDNA technology is a large step forward in detecting and profiling circulating biomarkers to derive clinically-relevant information. However, researchers and clinicians alike face challenges when attempting to extract high-quality cfDNA in a streamlined manner due to its low concentrations and short, fragmented nature. For efficient implementation of applications downstream, a high throughput method for purifying high-quality cfDNA is critical. To address these obstacles, Omega Bio-tek has developed a high throughput automated workflow configuration to extract cfDNA from ninety-six, 4 mL plasma samples in approximately 3 hours. The Mag-Bind® NAP STAR is an Assay-Ready Workstation specifically configured to run Omega Bio-tek's Mag-Bind® DNA purification Kits using Hamilton's Microlab® STAR™. Here, we elucidate the methods of the Mag-Bind® NAP STAR using Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298) and compare its performance to that of extractions performed manually using the same Kit.

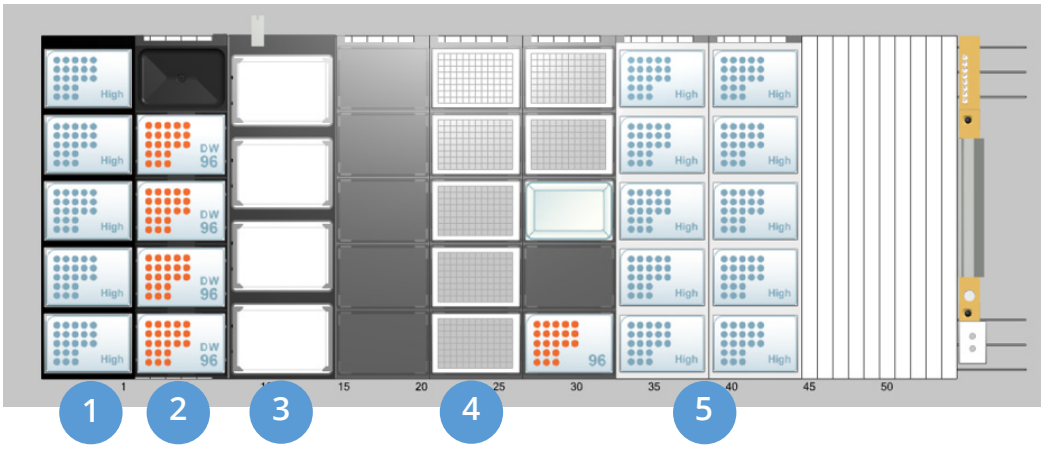
Materials and Methods

Omega Bio-tek's Mag-Bind® NAP STAR automates cfDNA purification from 4 mL of plasma on the Hamilton Microlab® STAR™. This protocol processes 1-4 sample plates and is compatible with common cfDNA collection tubes, such as Streck Cell-Free DNA BCT® or Roche Cell-Free DNA Collection Tubes. The workflow was automated using a Hamilton Microlab® STAR™; the components equipped, their purpose, and positions on the deck are mapped in **Figure 1.**

Three 4 mL aliquots were sampled from three lots of human-derived plasma for extractions using 4x24-well plates. Circulating cell-free DNA was extracted manually using Omega Bio-tek's Mag-Bind® cfDNA Kit following manufacturer's instructions. Following this, cfDNA was extracted using an automated protocol for 4x24-well plates on the Hamilton Microlab® STAR™ using the same Omega Bio-tek Kit.

cfDNA purified using both manual and automated protocols was analyzed on Agilent's TapeStation® 2200 for yield and quality. The samples extracted with the protocol utilizing 4x24-well plates were also subjected to qPCR for comparison to manual extraction methods and their efficacy in downstream applications.

Hamilton Microlab® STAR™ Deck Layout



Components	Purpose
1. Tip Isolator Carrier	For tips reused for tip mixing and liquid waste removal steps
2. MFX Carrier with 4x tall labware locators & liquid waste module for MPH	Magnet positions (Alpaqua Magnum FLX24) & liquid waste module
3. HHS Baseplate with 4x HHS units (3 mm orbital with flat bottom adapers)	Heater/Shakers for bead resuspension and incubation steps
4. 3x DWP Stands	For processing plate positions, reagent reservoirs, and elution plate
5. 2x Standard Tip Carriers	Tips for reagent dispenses

Figure 1. Hamilton Microlab® STAR™ deck layout for extraction of cfDNA from 4 mL plasma samples.

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Results and Discussion

cfDNA purified using both manual and automated extraction protocols were analyzed on Agilent's TapeStation® 2200 to derive information about the cfDNA's size and integrity **(Figures 2-4).** %cfDNA was determined by the TapeStation® 2200 analysis software. Across all three lots of plasma, the %cfDNA extracted using the Mag-Bind® NAP STAR was greater than or equal to %cfDNA extracted manually. **Figure 2** shows TapeStation® analysis of cfDNA extracted from plasma Lot A; these extractions produced a well-defined band at approximately 170 bp, the typical peak of cfDNA. **Figures 3 and 4** support the same conclusion, as each TapeStation® image illustrates proper migration with well-defined bands around 170 bp and additional multimers above.

TapeStation Analysis of cfDNA Purified from Plasma Lot A

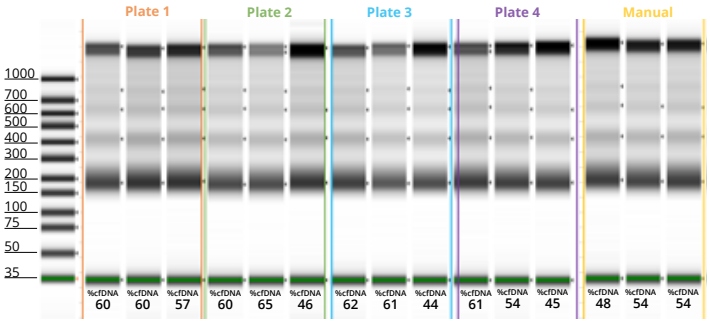

Figure 2. TapeStation Analysis performed on cfDNA extracted from three aliquots of 4 mL plasma sampled from Lot A showed well-defined bands.

TapeStation Analysis of cfDNA Purified from Plasma Lot B

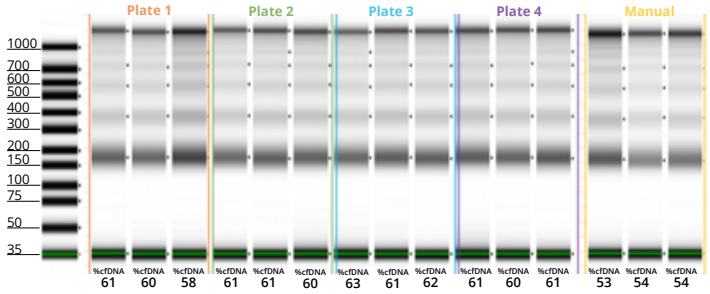

Figure 3. TapeStation Analysis performed on cfDNA extracted from three aliquots of 4 mL plasma sampled from Lot B showed successful cfDNA purification.

TapeStation Analysis of cfDNA Purified from Plasma Lot C

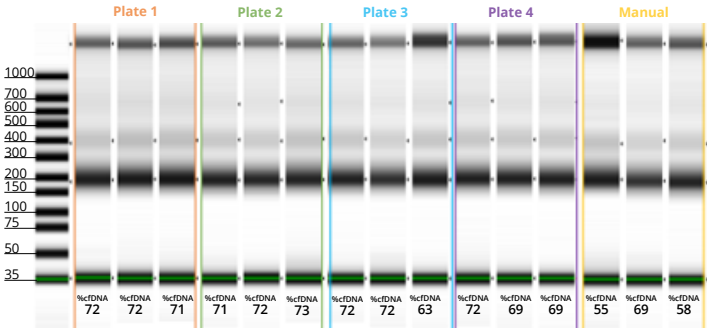

Figure 4. TapeStation Analysis performed on cfDNA extracted from three aliquots of 4 mL plasma sampled from Lot C showed well-defined bands and migration.

qPCR analysis was performed on the extracted cfDNA to determine its suitability for use downstream. **Figure 5** presents the ΔCt values obtained from this analysis. Because of the 2 µL and 6 µL template input volumes, the ΔCt's should theoretically be ~1.5. It can be seen in Figure 5 that extractions performed using Omega Bio-tek's automated protocol exhibit ΔCt's near the expected value of 1.5, indicating little to no inhibition; these results are also comparable to those obtained with manual extraction.

ΔCt Values from qPCR Analysis Illustrate Suitability for Downstream Applications

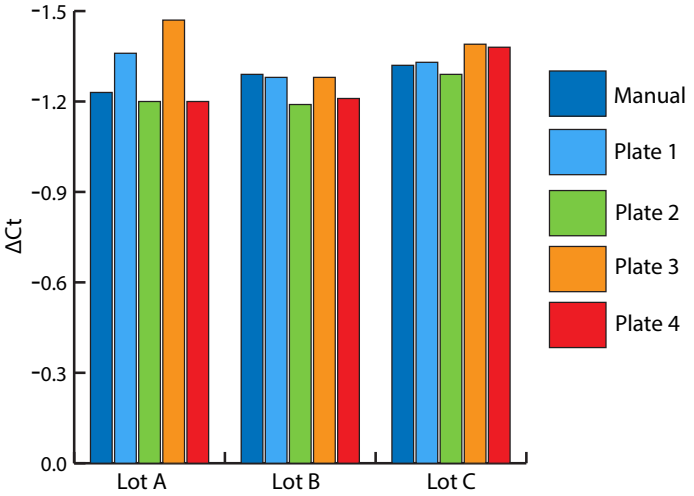

Figure 5. ΔCt values obtained from qPCR analysis of cfDNA extracted from each lot of plasma using both manual and automated methods.

Conclusions

Omega Bio-tek's Mag-Bind® NAP STAR offers an automated, high throughput purification solution for cfDNA from 4 mL plasma samples. This automation configuration produces high-quality cfDNA which is comparable to the cfDNA extracted using the manual protocol. Using this workflow, ninety-six 4 mL plasma samples can be processed in approximately 3 hours, starting with samples already transferred to the 24-well plate. The high yield and quality of the purified cfDNA support its suitability for use in downstream applications, such as qPCR.

Product Information

Product No.	Description
M3298-00 M3298-01 M3298-02	**Mag-Bind® cfDNA Kit**

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