

cfDNA Purification from Different Blood Collection Tubes: A Compatibility Study of Omega Bio-tek's Extraction Chemistry on the MagBinder® Fit²⁴ Platform

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Introduction

Cell-free DNA (cfDNA) has emerged as a tool of great potential in molecular diagnostics because of its ability to provide a snapshot of the epigenetic signature of its tissue of origin with minimally-invasive sample collection. However, cfDNA has not reached its peak utilization due to several hurdles obstructing high-quality extraction. The low concentrations in which cfDNA is found and the large amount of cfDNA needed for applications downstream are some of these hurdles. Because of the extremely low concentrations in which cfDNA is present, pre-analytical sample storage and stabilization are of the utmost importance to minimize cfDNA degradation and ensure high-quality extractions. Here, we validate and showcase the compatibility of Omega Bio-tek's chemistry for extraction of cfDNA from blood samples stored in some of the most common blood collection tubes on the market for cfDNA stabilization purposes.

cfDNA extraction was achieved by employing Omega Bio-tek's pre-filled MB Fit²⁴™ cfDNA Kits (B3298-10-48PF) automated on the MagBinder® Fit²⁴ Nucleic Acid Purification System. The MagBinder® Fit²⁴ is a nucleic acid purification system designed to meet the needs of low-to-moderate throughput users by providing semi-automated purification of 1 to 24 small or large volume sample inputs. The pre-filled nature of the MB Fit²⁴™ cfDNA Kit enhances ease of use, convenience, and extraction accuracy and reduces hands-on time by skipping reagent preparation and buffer dispensing steps.

Materials and Methods

Sample Collection and Storage

Four different blood collection tubes were included in this compatibility study: Roche Cell-Free DNA Collection Tube, Streck Cell-Free DNA BCT, Biomatrix LBgard Blood Tube, and Mawi HemaSure-OMICS. Blood collected in each collection tube was left to sit at room temperature for 24 hours. Plasma was derived based on manufacturer's instructions and stored at -20°C until used for cfDNA extraction. Blood-derived K3EDTA plasma served as the extraction control.

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 from each of the collection tubes (n = 5) 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, Quality, and Downstream Performance

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. cfDNA purified from 4 mL working volumes were subjected to qPCR at two different template amounts, 2 µL and 6 µL to evaluate its performance downstream.

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

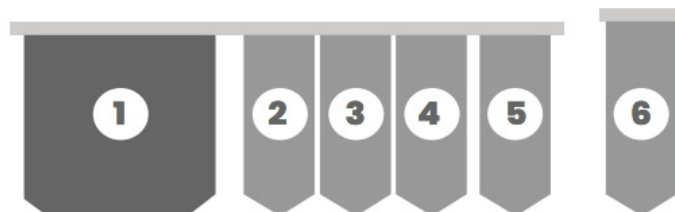


Figure 1. MB Fit²⁴™ cfDNA Kit reagent cartridge positions, contents, and volumes.

MagBinder® Fit²⁴



Results and Discussion

TapeStation Analysis of Purified cfDNA from Leading Blood Collection Tubes

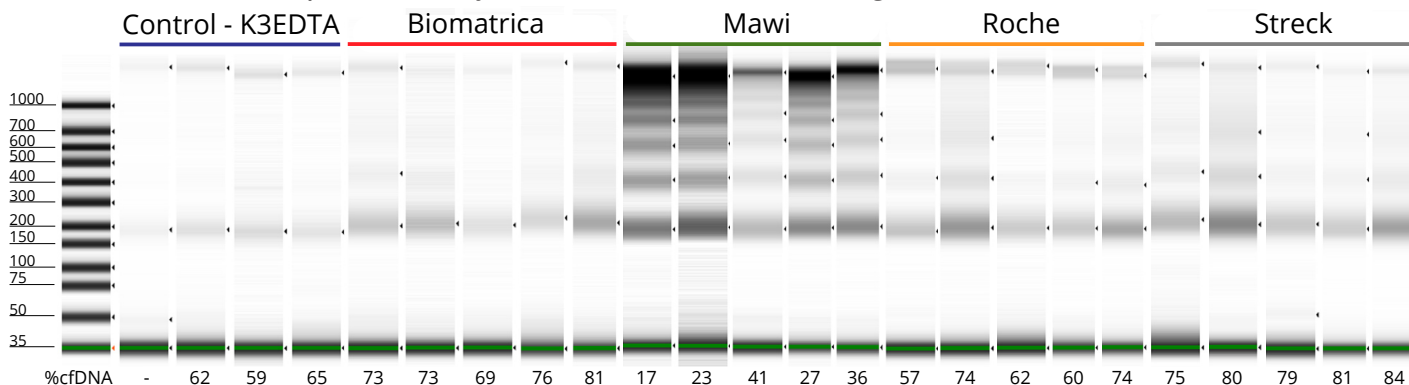


Figure 2. cfDNA extracted from plasma stored in four types of blood collection tubes that stabilize cfDNA. cfDNA was extracted using the MB Fit24™ cfDNA Kit on the MagBinder® Fit24 platform.

TapeStation analysis using the Cell-Free DNA ScreenTape Assay was performed to evaluate the yield and quality of extracted cfDNA from sample inputs stored in 4 different brands of collection tubes. Bands concentrated between 150 and 200 base pairs indicate high yielding cfDNA (Figure 2), validating the compatibility of Omega Bio-tek’s extraction chemistry with all blood collection tubes tested. Here, the samples labeled K3EDTA represents blood-derived K3EDTA plasma which served as the control. The remaining samples were stored in tubes from Biomatrixa, Mawi, Roche, and Streck. The highest yields of cfDNA were extracted from Mawi, Streck, and Roche, respectively.

Conclusions

Optimal sample storage conditions and reliable extraction methodology are integral to fully harnessing the potential of cfDNA. Here, we illustrated the compatibility of the MagBinder® Fit24 platform with four of the leading blood collection tubes. The semi-automated cfDNA extraction workflow 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 from all four blood collection tubes tested.

Product Information

Product	Description
MB Fit24™ cfDNA Kit	B3298-10-48PF
MB Fit24™ cfDNA Kit CE IVD	B3298-10-48PFCEIVD
MagBinder® Fit24	B1-001-24
MagBinder® Fit24 CE IVD	B1-001-24CEIVD

Average Ct from qPCR Analysis from Different Blood Collection Tubes

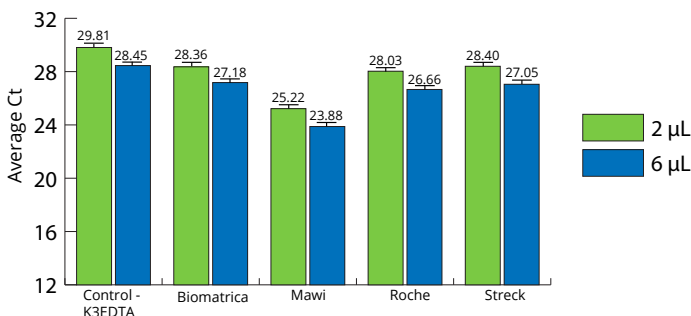


Figure 3. Average Ct values obtained from qPCR analysis of cfDNA extracted from plasma stored in each type of collection tube.

qPCR analysis was performed on the extracted cfDNA to determine its suitability for use downstream. Figure 3 presents the average Ct values obtained from each type of collection tube. The expected ΔCt should be ~1.5 based on the 2 µL and 6 µL template amounts used. The average Ct values are ~1.4 for Roche, Streck, and Mawi’s collection tubes and ~1.2 from Biomatrixa’s tube, exhibiting successful downstream qPCR performance with minimal inhibition.