Automated Cell-Free DNA Purification from 10 mL Plasma with the Dynamic Devices Lynx®

Purify cell-free DNA from 10 mL plasma with Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298) on the Dynamic Devices Lynx® robotic liquid handler.

Kit

Mag-Bind® cfDNA Kit (M3298)

Sample Type

Plasma collected in Streck Cell-Free DNA BCT

Sample Amount

10 mL

Analysis

qPCR, TapeStation®

Materials

Mag-Bind® cfDNA Kit (M3298)
Dynamic Devices Lynx® LM1200 Robotic Liquid Handler

- 24 ST Pipetting Tool
- 24XL MagRod Tool
- iMagZ Magnet Elevator
- Bioshake D30T-Elm
- Source Tube Holders

Alpaqua FLX HV 24 Magnet (Alpaqua, Cat.# A000640)

For a complete list of system components and plastic consumables on Dynamic Devices Lynx®, please contact us at automation@omegabiotek.com

Methods

Automated method developed by Omega Bio-tek for the Dynamic Devices Lynx®
24 samples extracted in ~1.5 hours

Multiple plates can be processed with additional equipment in development from Dynamic Devices and would change timing

innovations in nucleic acid isolation

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Reagent Setup & Deck Layout

Reagent Container	Reagent	Volume per Sample	Volume Required for 24 Samples†
E-Z Select® Robotic Reservoir, 600 mL	JSB Binding Buffer + Mag-Bind® Particles CH‡	10 mL + 50 µL	270 mL + 1.35 mL
4 Column Trough (position 3)	Proteinase K	200 µL	5.3 mL
4 Column Trough (position 4)	DS Buffer	670 µL	21 mL
E-Z Select® Robotic Reservoir, 600 mL	GT7 Wash Buffer	4 mL	125 mL
E-Z Select® Robotic Reservoir, 600 mL	SPW Wash Buffer	4 mL	125 mL
300 mL Reservoir	Elution Buffer	75-200 µL	60 mL

†Indicated volumes include required dead volume for reagent troughs.
‡To be prepared as a master mix.



Position	Hardware/Consumable
A1	24 Tip 5 mL Pipette Core
A6	Bioshake D30T-Elm
B3, B4	Sample Input Tubes
B6	E-Z Select® 24-Well Plate, 25 mL
C1, D1, E1	DDX 24 5 mL Tips Filtered (Dynamic Devices, Cat.# DDX-24-5000F-1)
C6	XL Reservoir for Liquid Waste
D2	Proteinase K + DS Buffer
D3	JSB Binding Buffer + Mag-Bind® Particles CH
D4	GT7 Wash Buffer
D5	SPW Wash Buffer
D6	Elution Buffer
F2, F3, F4, F5, F6	24 MagRod Well Plate (Dynamic Devices, Cat.#MKS-24-DWP-1)
G1	24 XL MagRod Tool

Figure 1. Illustration of the deck layout for the Dynamic Devices Lynx® LM1200 configured for cfDNA extraction from 10 mL plasma.

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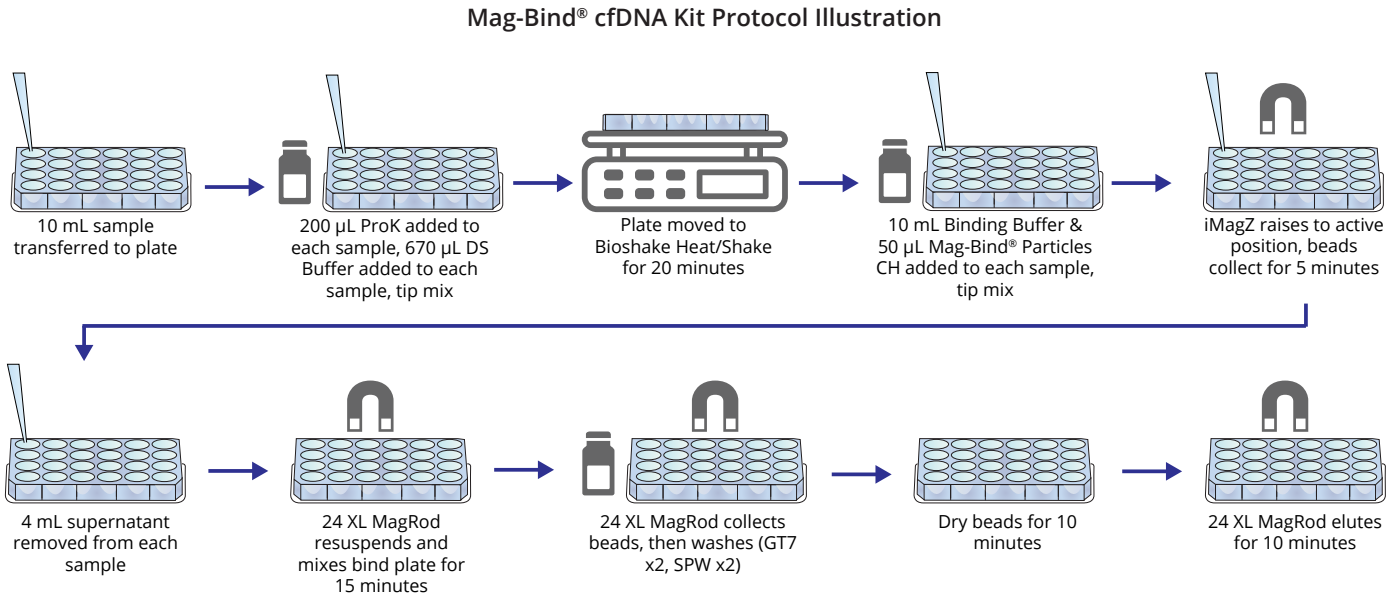

Figure 2. Workflow illustration of cfDNA extraction using the Mag-Bind® cfDNA Kit (M3298) automated on the Dynamic Devices Lynx® LM1200.

Methods

The workflow and protocol described above (**Figures 1 & 2**) were used to purify cell-free DNA from 10 mL of plasma. Results were compared to a control which followed the same protocol manually. In addition, two elution volumes were tested, 75 µL and 200 µL. cfDNA from the 75 µL elution protocol was quantified via TapeStation® and subjected to qPCR analysis. cfDNA from the 200 µL elution protocol was also quantified via TapeStation®.

Results

cfDNA was purified in triplicate from three different 10 mL plasma samples, eluted in 75 µL, and quantified via Agilent's TapeStation® 4150 (**Figure 3**). As expected, yields between the manual and automated protocols were comparable. qPCR analysis was also performed on purified cfDNA using 2 µL and 6 µL input amounts of template DNA to evaluate the presence of PCR inhibitors. The average ΔCt between the input amounts of manually purified cfDNA was below the expected 1.5 value, indicating the presence of inhibitors, whereas the average ΔCt between inputs of automated samples aligned with the expected value of 1.5, indicating the samples were pure and contained minimal traces of inhibitors (**Table 1**). There was no detectable fluorescence in the no template controls.

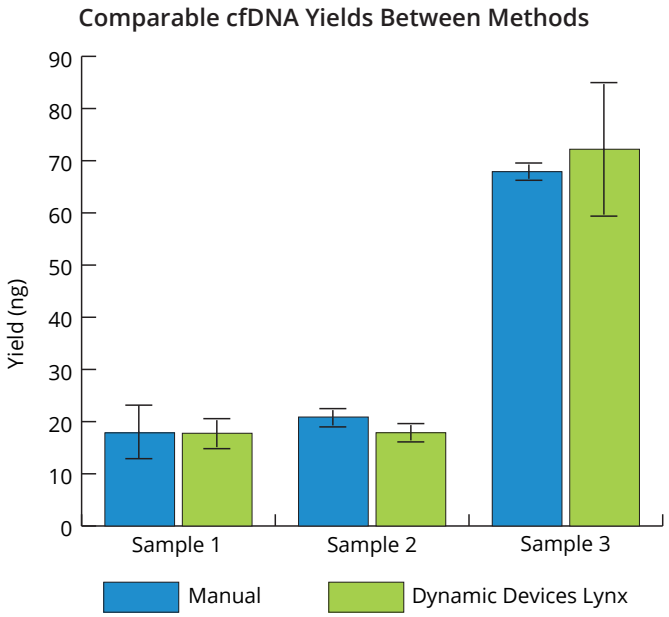

Figure 3. Average cfDNA (50-700 bp) yield values from 10 mL plasma samples eluted in 75 µL.

Table 1. Average Ct values from qPCR analysis of purified cfDNA eluted in 75 µL.

Sample	Manual			Dynamic Devices Lynx®		
	2 µL	6 µL	ΔCt	2 µL	6 µL	ΔCt
1	29.12 ± 0.33	28.27 ± 0.38	0.85	29.28 ± 0.48	27.73 ± 0.50	1.55
2	29.51 ± 0.28	28.27 ± 0.10	1.24	29.64 ± 0.17	28.22 ± 0.16	1.42
3	28.39 ± 0.30	27.73 ± 0.37	0.67	28.52 ± 0.25	26.96 ± 0.14	1.56

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Table 2. Quantification data of cfDNA purified via automated and manual means and eluted in 200 µL. Yields were comparable between purification methods.

Sample ID	% cfDNA	50 - 700 bp Nucleic Acid Concentration (pg/µL)	Yield (ng)	Average Yield (ng)
Manual	60	297	59.40	59.10
	60	294	58.80	
Dynamic Devices Lynx®	68	295	59.00	58.53
	68	293	58.60	
	69	290	58.00	

Comparable cfDNA Yields and Integrity Between Purification Methods

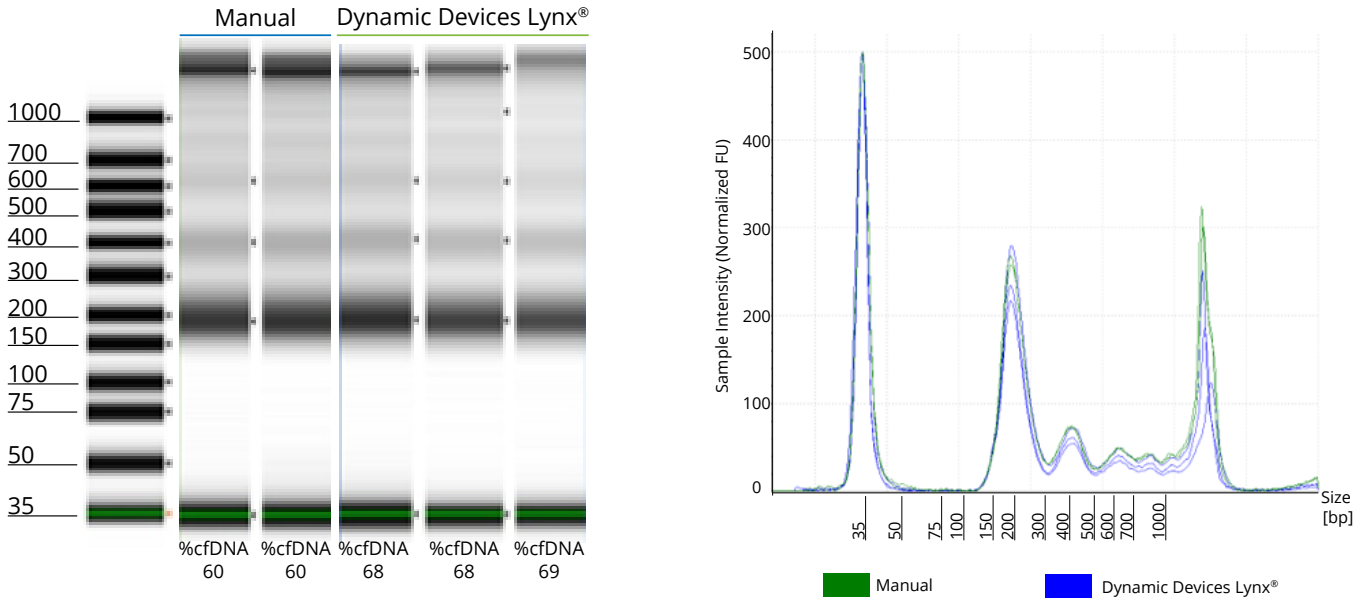

Figure 4. TapeStation® analysis illustrates comparable cfDNA yields and integrity between automated and manual purification methods when eluting in 200 µL.

Subsequently, cfDNA was purified from 10 mL plasma samples using the same protocol, but the elution volume was raised to 200 µL. cfDNA was again quantified via TapeStation®, and, again, cfDNA yields were comparable between automated and manual purification methods (**Table 2**). Integrity analysis via TapeStation also reveals comparable dark bands of cfDNA in the targeted region between 50 - 700 bp (**Figure 4**).

Conclusions

Omega Bio-tek and Dynamic Devices have collaborated to create a robust method for automating cfDNA purification from high-volume plasma samples up to 10 mL. This workflow eliminates upfront manual processing and the need for sample splitting across multiple plates, providing a true walk-away solution. cfDNA purified using this automated workflow is shown to be equivalent or better in terms of yield and purity compared to cfDNA purified from manual techniques.

Product Information

Description	Product No.	Size
Mag-Bind® cfDNA Kit	M3298-00	5 Preps
	M3298-01	50 Preps
	M3298-02	200 Preps

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