



A workflow for medium-throughput isolation of cfDNA from plasma samples using Apostle MiniMax™ on the KingFisher™ Technology

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Summary

Cell-free DNA (cfDNA) is found in various bodily fluids. cfDNA has a characteristic size of approximately 175 bp. Due to its small size and low quantity the main challenge is to get enough cfDNA for sequencing. Subsequently, this results in a need to extract from larger volumes of bodily fluid, yet higher input volumes can be more difficult to manage for high-throughput sample processing.

This application note demonstrates the use of Apostle MiniMax™ High Efficiency cfDNA Isolation Kit, in conjunction with the KingFisher Duo Prime automated protein purification system. This potential solution that mitigates some of the challenges of processing large volume samples. Automating the chemistry can also reduce the risk of human error, reduce hands-on time and total time; therefore giving the user the ability to run more samples in a day.

Materials and Methods

Extraction of cfDNA from 1 mL and 2 mL plasma collected in EDTA tubes was semi-automated on the KingFisher™ Duo Prime using Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Standard Edition). Samples were stored at -80°C for >6 months and extractions took place during that time span. Prior to extraction, the plasma samples were thawed at room temperature. The plasma samples were first processed manually for lysis following the steps detailed in the protocol followed by cfDNA purification on the KinFisher Duo Prime. The second set of samples was extracted following a completely manual extraction following the Apostle MiniMax™ protocol.

Manual processing

In this study, only one donor was used to assess the differences between manual and semi-automated cfDNA extraction. This donor was chosen due to previous work showing that this plasma sample had measurable quantities of cfDNA. Sample lysis buffer and proteinase K were added to the plasma sample. The sample was incubated at 60°C for 20 minutes. The tubes were cooled at the end of the incubation to room temperature. The cfDNA Lysis/Binding Solution and Magnetic Nanoparticles were added to the sample followed by a 10 minute incubation at room temperature while shaking. The plate was then moved to the KingFisher™ Duo Prime for further extraction.

Automated processing

The parameters for mix times and speeds and the collect times were optimized for the magnetic beads on the KingFisher™ Duo Prime. The optimized parameters and reagent volumes for a 1 mL sample of plasma collected in an EDTA tube can be seen in Table 1 and for a 2 mL sample of plasma collected in an EDTA tube can be seen in Table 2.

DNA Purification Step	Plate	Plate Row	Reagent	Volume (µL)	Automation parameters	
					Mixing time/ Mixing Speed	Collect Count/ time [s]
Bind	1	A	Lysate	1140	1 min/ Medium	8/ 30 sec
			cfDNA Lysis/Binding Solution	1250		
			Magnetic NanoParticles	15		
Wash 1	1	B	cfDNA Wash Solution	1000	1 min/ Medium	3/30sec
Wash 1	1	C	cfDNA Wash Solution	1000	1 min/ Medium	3/30sec
NA	1	D	KingFisher™ Duo 6-Tip Comb	NA		
Wash 2	2	A	cfDNA 2 nd Wash Solution	200	1 min/ Medium	5/30sec
			100% Ethanol	800		
Wash 2	2	B	cfDNA 2 nd Wash Solution	200	1 min/ Medium	5/30sec
			100% Ethanol	800		
NA	2	C	NA	NA		
Elution	2	D	cfDNA Elution Solution	20	1 min/ Medium	3/30sec

Table 1. KingFisher™ Duo Prime parameters for cfDNA extraction from a 1 mL plasma sample.

DNA Purification Step	Plate	Plate Row	Reagent	Volume (µL)	Automation parameters	
					Mixing time/ Mixing Speed/ Pause Time	Collect Count/ time [s]
Bind	1	A	Lysate	2280	1 min/ Medium	8/ 30 sec
			cfDNA Lysis/Binding Solution	2500		
			Magnetic NanoParticles	30		
Wash 1	1	B	cfDNA Wash Solution	1000	1 min/ Medium	3/30sec
Wash 1	1	C	cfDNA Wash Solution	1000	1 min/ Medium	3/30sec
NA	1	D	KingFisher™ Duo 6-Tip Comb	NA		
Wash 2	2	A	cfDNA 2 nd Wash Solution	200	1 min/ Medium	5/30sec
			100% Ethanol	800		
Wash 2	2	B	cfDNA 2 nd Wash Solution	200	1 min/ Medium	5/30sec
			100% Ethanol	800		
NA	2	C	NA	NA		
Elution	2	D	cfDNA Elution Solution	40	1 min/ Medium	3/30sec

Table 2. KingFisher™ Duo Prime parameters for cfDNA extraction from a 2 mL plasma sample.

Results

The manual extractions were compared to the extractions done on the KingFisher™ Duo Prime. The total yields were compared and presented in Figure 1. The yield of DNA was estimated by using Quant-it™ dsDNA Assay Kit. The yield varied for the manual and automated extraction for the two different input volumes due to experimental variation. The coefficient of variation was calculated for the 4 different groups (Table 3). For both of the plasma volumes the coefficient of variation for the automated methods was less than for the manual method indicating that there is less variability in relation to the average yield when processing on the KingFisher™ Duo Prime.

Due to the volume limitations on the KingFisher™ Duo Prime the largest input sample volume is 2 mL. If a larger volume is desired the sample volumes can be split between multiple wells and the elution can be combined.

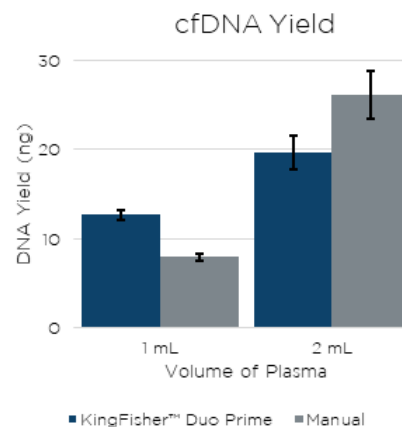


Figure 1. The cfDNA extracted using the Apostle MiniMax™ on KingFisher™ Duo Prime and by Manual extraction. The error bars are representative of the standard deviation of three technical replicates.

Volume of Plasma	KingFisher™ Duo Prime	Manual
1 mL	4.4%	5.0%
2 mL	9.6%	10.0%

Table 3. The coefficient of variation for the 4 different conditions

Conclusions

Using a semi-automated platform it is demonstrated to reduce hands-on-time significantly without sacrificing cfDNA yield (table 3). Automation can also help reduce the risk for human errors as there are less human intervention steps. The throughput of the KingFisher™ Duo Prime is only 6 samples per run due to the large volumes required to extract sufficient cfDNA from plasma. The overall protocol does not require significant time, and at max capacity in an 8 hour day, 36 samples can be processed with only 30 minutes of total hands-on-time.

A supplemental protocol is also available for processing a 2 mL sample with the KingFisher™ Flex.

	Throughput per run	Hands-on Time	Walk away Time	Total Time
Manual	24	45 minutes	30 minutes	75 minutes
Semi-automated KingFisher™ Duo	6	5 minutes	70 minutes	75 minutes

Table 4. The throughput and time for manual and semi-automated cfDNA extraction

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