BECKMAN COULTER
Life Sciences

Cell-Free DNA (cfDNA) Isolation from Plasma using Apostle MiniMax™ High Efficiency cfDNA Isolation Kit on a King Fisher™ Duo Prime

This method is applicable for the extraction of cfDNA from plasma using a semi-automated solution.

Please reference current Apostle MiniMax™ High Efficiency cfDNA Isolation Kit protocol for product information (Part Number - C40605, C40604, C40603)

Purpose

This protocol demonstrates the Apostle MiniMax High Efficiency cfDNA Isolation Kit performance on the KingFisher Duo Prime purification system. Automation serves as a solution that mitigates the challenges of processing large volume (greater than 1mL) samples. Automating the chemistry can also reduce the risk of human error, reduce hands-on time (HOT) and total turn-around time (TAT); therefore giving the user the ability to run more samples per day.

Materials Used

Material	Part Number	Supplier
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AB02123	AmericanBio
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
KingFisher™ Duo 6 Tip Comb and 24 Deepwell Plates	97003510	ThermoFisher Scientific
10 mL 24 Well Plate	7701-5102	GE Healthcare Life Sciences

KingFisher Duo Prime Parameters

DNA Purification Step	Plate	Plate Row	Reagent	1 mL Sample Volume (µL)	2 mL Sample Volume (µL)	Automation parameters	
						Mixing time/ Mixing Speed	Collect Count/ time [s]
Bind	1	A	Lysate	1120	2260	1 min/ Medium	8/ 30 sec
			cfDNA Lysis/Binding Solution	1250	2500		
			Magnetic NanoParticles	15	30		
Wash 1	1	B	cfDNA Wash Solution	1000	1000	1 min/ Medium	3/30sec
Wash 1	1	C	cfDNA Wash Solution	1000	1000	1 min/ Medium	3/30sec
NA	1	D	KingFisher™ Duo 6-Tip Comb	NA	NA		
Wash 2	2	A	cfDNA 2nd Wash Solution	250	250	1 min/ Medium	5/30sec
			100% Ethanol	750	750		
Wash 2	2	B	cfDNA 2nd Wash Solution	250	250	1 min/ Medium	5/30sec
			100% Ethanol	750	750		
Wash 1	2	C	NA	NA	NA		
Elution	2	D	cfDNA Elution Solution	20	40	1 min/ Medium	3/30sec

Table 1. KingFisher™ Duo Prime parameters for cfDNA extraction from a 1 and 2 mL plasma sample.

Protocol

The instruction below is for 1 mL sample volume. If processing 2 mL of plasma, double the volumes of each of the reagents.

1. **Lysis**
    a. Centrifuge samples at **9500 RPM** for **10 minutes**
    b. Transfer **1 mL** of **sample** to 24 well plate
    c. Add **40 µL** of **Proteinase K** to 24 well plate
    d. Add **100 µL** of **Sample Lysis Buffer** to 24 well plate
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. **Incubate** the plate for **20 minutes** at **60°C**
2. **Bind**
    a. Vortex the tube of **Magnetic Nanoparticles** to fully resuspend the beads
    b. Prepare the **binding/nanoparticle solution** in a tube
        i. Add **1.25 mL** of **cfDNA Lysis/Binding Solution**
        ii. Add **15 µL** of **Magnetic Nanoparticles**
    c. Vortex the tube of **binding/nanoparticle solution** to fully suspend
    d. Add **1.265 mL** of **binding/nanoparticle solution** to the 24 well plate
    e. **Incubate** the plate for **10 minutes** at **room temperature** while **shaking** at moderate-high speed
    f. **Transfer** the sample to Row A of the KingFisher 24-well plate
3. **Automated Processing**
    a. Load the KingFisher with Apostle MiniMax™ kit for 1 mL plasma/serum script
    b. Press the **START** button
    c. Insert the plates into the KingFisher as the instruments instructs
4. **Elute**
    a. After the KingFisher™ Duo Prime is complete, remove plate
    b. Transfer and **Save** the supernatant in Plate 2 Row D to a plate for storage

Example Data

In this study, one donor was used to assess the differences between the manual and the semi-automated cfDNA extraction. The donor selected had previously been used in a study that indicated this plasma sample had measurable quantities of cfDNA. Three replicates of the sample were run for each extractions method. Figure 1 shows the yield comparison by Quant-it™ dsDNA Assay Kit. Differences in sample yield is a result of experimental variation. Shown in table 3 is the calculation of the coefficient of variation (%cv) for the four different conditions. The automated %cv of the plasma was slightly lower than for the manual method, indicating less variability in average yield.

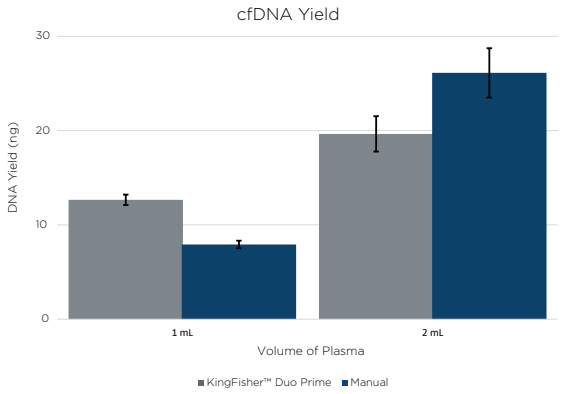

Figure 1. The cfDNA extracted using the KingFisher and by Manual extraction. The error bars are representative of the standard deviation of three technical replicates.

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

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