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Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual (Type S), 25-50 Preps

– designed for small DNA (<100bp) fragments

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Product description

The Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Type S) is designed for isolation of DNA from cell free plasma, serum, urine samples. The kit is featured for its efficient recovery of small DNA fragments (<100 bp) from biological samples. The kit uses proprietary Apostle MiniMax™ technology, offers highly efficient, reproducible recovery of high-quality cfDNA with high yield. The isolated DNA samples is suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

Kit capacity

The kit is capable of cfDNA isolation for 25-50 samples, depend on sample volume.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles (S)	1.5 mL	2-8^{0}C
cfDNA Lysis/Binding Solution (S)	125 mL	Room Temperature, in dark
cfDNA Wash Solution (S)	70 mL	
cfDNA 2nd Wash Solution (S)	25 mL	
cfDNA Elution Solution (S)	1.5 mL	

Note: Upon receival of the kit, check component to make sure the components are not frozen due to shipping. If the components are frozen, thaw all component before use. May need to sonicate the nanoparticle solution to resuspend the particle after freeze and thaw.

Required materials not supplied

Adjustable micropipetteors (1 mL, 200 uL, 20 uL) and tips
Magnets (Magnets specifically designed for 15 mL and 2 mL tubes are preferred)
Table top centrifuge
Nonstick, DNase/RNase-free tubes (1.5 mL, 15 mL, 50 mL)
Vortex
Shaker
Ethanol, 200 proof
Heater (for sample lysis)
Proteinase K, 20mg/ml
20% SDS solution
Isopropanol, 100%

Procedure for manual isolation of cfDNA

A. Reagent Preparation

1. Add 35 mL Isopropanol to the cfDNA Wash Solution (S) bottle, to a final volume of 105 mL, and mix well.

Caution: Make sure to follow the Reagent Preparation step before experiment. Check the box on the bottle label to reflect the completion of reagent preparation.

B. Sample lysis (Note: Optional. **Required** if you collect your samples in Streck Cell-Free DNA BCT tubes, or other blood collection systems which may cause crosslinking of DNA to other biomolecules; **Preferred** if using automated cfDNA extraction system)

2. Add components to a tube (15 mL tube for sample volume ≤ 4mL, 50 mL tube for sample volume > 4mL) **in the order** indicated below, based on volume of sample.

Reagents	Plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
Plasma/serum	1 mL	2 mL	4 mL	8 mL
Proteinase K, 20mg/ml	15 uL	30 uL	60 uL	120 uL
20% SDS solution	50 uL	100 uL	200 uL	400 uL

Caution: avoid mixing proteinase K with SDS before Plasma/serum.

3. Mix the solution well (invert the tube 10 times, or vortex briefly) and incubate the mixture at 60ºC for 20 minutes.

4. At the end of the incubation, cool the tubes containing the plasma to room temperature (e.g. put the tube in ice water for 5 min).

C. Bind cfDNA to magnetic nanoparticles

5. Prepare the binding/nanoparticle solution according to the table below, and mix well (**Note**: vortex the Apostle MiniMax™ Magnetic Nanoparticles (S) vial (White Cap) to fully resuspend the nanoparticles before use):

Reagents	Plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
cfDNA Lysis/Binding Solution (S)	1.25 mL	2.5 mL	5 mL	10 mL
Magnetic Nanoparticles (S)	15 uL	30 uL	60 uL	120 uL

6. Add the prepared binding/nanoparticle solution to the plasma/serum sample, thoroughly mix by vortexing briefly, or invert the tube 10 times (**Note**: avoid excessive vortexing, which generate excessive bubbles).

7. Shake at moderate-high speed for 5 minuets to bind the cfDNA to the nanoparticles.

8. Add Isopropanol (100%) to the sample mixture according to the table below:

Reagents	Initial plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
Isopropanol	0.75 mL	1.5 mL	3 mL	6 mL

9. Shake at moderate-high speed for another 5 minuets.

10. Place the tube on magnet for 5 min, or until the solution clears and the beads are pelleted against the magnet.

11. Carefully remove the supernatant (e.g. using pipette to remove supernatant, or discard the supernatant with the existence of the magnet to attract nanoparticles).

D. Wash with Apostle MiniMax™ cfDNA Wash Solution

12. Remove the tube from the magnet, add 1 mL of prepared Apostle MiniMax™ cfDNA Wash Solution (S), vortex to resuspend the nanoparticles.

13. Transfer the magnetic nanoparticle suspension to a new non-stick 1.5 mL microcentrifuge tube, and save the lysis/binding tube.

14. Place the 1.5 mL tube on magnet to pellet the nanoparticles for 1 min.

15. Use the supernatant in the 1.5 mL tube to rinse the saved lysis/binding tube, and transfer any residual nanoparticles to the 1.5mL tube, discard the lysis/binding tube.

16. Place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

17. Remove the supernatant carefully using pipette.

18. Remove the 1.5 mL tube from the magnet, add 1 mL of Apostle MiniMax™ cfDNA Wash Solution (S), then vortex for 30 seconds.

19. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

20. Remove the supernatant carefully using pipette.

E. Wash with Apostle MiniMax™ cfDNA 2nd Wash Solution

21. Pre-dilute Apostle MiniMax™ cfDNA 2nd Wash Solution 1:4 in Ethanol before use, to a final composition of 20% Apostle MiniMax™ cfDNA 2nd Wash Solution and 80% Ethanol.

    The amount of final secondary wash buffer required is 2mL per sample.

22. Remove the 1.5 mL tube from the magnet, add 1 mL of the prepared Apostle MiniMax™ cfDNA 2nd Wash Solution (S) (1:4 diluted in ethanol), then vortex for 30 seconds.

23. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

24. Remove the supernatant carefully using pipette.

25. Repeat step 22-24 for a second wash.

26. Keep the 1.5 mL tube on the magnet, air dry the nanoparticles for 3 minutes.

27. Carefully remove the residual supernatant using pipette.

F. Elute cfDNA from magnetic nanoparticles

28. Remove the 1.5 mL tube from the magnet, add Apostle MiniMax™ cfDNA Elution Solution (S) (**Blue Cap**) to the 1.5 mL tube according to the following table, based on initial sample volume.

Reagents	Plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
cfDNA Elution Solution (S)	15 uL	30 uL	50 uL	100 uL

29. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 minutes to elute the cfDNA from the nanoparticle.

30. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on a magnet, until the solution clears and the nanoparticles are pelleted against the magnets.

31. Collect the supernatant that contains cfDNA in a non-stick, DNase and RNase free microcentrifuge tube.

32. Store the cfDNA sample at 4^0C for short term storage, and -20 ^{0}C for long term storage.

33. If isolated cfDNA sample characterization and quantification is needed, it is recommended to use Bioanalyzer 2100 + High Sensitivity DNA Analysis Kit, due to its low detection limit (5pg/ul).