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Apostle MiniMax™ High Efficiency cfDNA Isolation Kit Manual (Type S), 5-10 Preps

– designed for small DNA (<100bp) and single strand DNA fragments

Cat#: A17830-10, Version: P.20

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) and single strand DNA from biological samples. The kit uses proprietary Apostle MiniMax™ technology, offers highly efficient 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 5-10 samples, depend on sample volume.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles (S)	0.3 mL	2-8^{0}C
cfDNA Lysis/Binding Solution (S)	20 mL	Room Temperature, in dark
Protein Precipitation Solution (S)	2 mL	
cfDNA Wash Solution (S)	20 mL	
cfDNA Elution Solution (S)	0.6 mL	

Note: Magnetic nanoparticle solution should be brown solution. Vortex magnetic nanoparticle solution to fully resuspend the nanoparticles before use.

All other solutions stored at room temperature (15-30^{0}C) should be clear solution. If precipitate is observed in any reagent, warm the solution to 37^{0}C until the precipitate dissolves.

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)
Centrifuge (12,000g), Table top centrifuge
Heater (for sample lysis)
Nonstick, DNase/RNase-free tubes (1.5 mL, 15 mL, 50 mL)
Vortex, Shaker
Ethanol, 200 proof
Isopropanol, 100%
Ultrapure, DNase/RNase free water
Proteinase K, 20 mg/mL

Procedure for manual isolation of cfDNA

A. Sample treatment

1. Add components to a tube (1.5 mL microcentrifuge tube for 1 ml sample, 15 mL tube for sample volume > 1 mL) **in the order** indicated below, based on volume of sample.

Reagents	Plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
Proteinase K, 20 mg/mL	20 uL	40 uL	80 uL	160 uL
Plasma/serum	1 mL	2 mL	4 mL	8 mL
cfDNA Lysis/Binding Solution (S)	100 uL	200 uL	400 uL	800 uL

Caution: avoid mixing proteinase K with cfDNA Lysis/Binding Solution (S) before Plasma/serum.

2. Mix the solution well by vortexing briefly and incubate the mixture at 60ºC for 20 minutes.

3. At the end of the incubation, cool the tubes containing the plasma/serum to room temperature.

4. Add Protein Precipitation Solution (S) (**Yellow Cap**) to the mixture, based on the sample volume indicated below. Vortex for 20 seconds, make sure the precipitation is uniformly dispersed.

Plasma/serum volume	1 mL	2 mL	4 mL	8 mL
Protein Precipitation Solution (S)	60 uL	120 uL	240 uL	480 uL

5. Centrifuge the mixture for 3 minutes at 12,000g to pellet the precipitate. The supernatant should be clear.

Note: If centrifuge with 12,000g capacity is not available for 15 ml conical tubes (for sample volume > 1mL), aliquot sample into 1.5 mL tubes and perform step 1-5 for all the aliquots. For example, 4 mL plasma can be aliquoted into four 1.5 mL tubes (1mL plasma per tube), followed by 1mL sample treatment protocol for each tube. The supernatant from each tube can then be combined for subsequent cfDNA isolation based on initial sample volume (4mL plasma in this example).

B. Bind cfDNA to magnetic nanoparticles

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

Reagents	Initial plasma/serum volume			
	1 mL	2 mL	4 mL	8 mL
cfDNA Lysis/Binding Solution (S)	0.9 mL	1.8 mL	3.6 mL	7.2 mL
Magnetic Nanoparticles (S)	15 uL	30 uL	60 uL	120 uL
Isopropanol (100%)	1.5 mL	3 mL	6 mL	12 mL

7. Add the supernatant from step 5 (~ 1 ml supernatant for each 1 ml initial plasma/serum) to the prepared binding/nanoparticle solution, thoroughly mix by vortexing briefly, or invert the tube 10 times.

8. Shake at moderate-high speed for 10 minutes to bind the cfDNA to the nanoparticles.

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

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

C. Wash with Apostle MiniMax™ cfDNA Wash Solution

11. Remove the tube (referred to as lysis/binding tube below) from the magnet, add 1 mL of Apostle MiniMax™ cfDNA Wash Solution (S), vortex to resuspend the nanoparticles.

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

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

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

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

16. Remove the supernatant carefully using pipette.

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

18. 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.

19. Remove the supernatant carefully using pipette.

D. Second Wash with 80% Ethanol

20. Remove the 1.5 mL tube from the magnet, add 1 mL 80% Ethanol (made by mixing pure ethanol with ultrapure & DNase/RNase free water, at 4:1 ratio), then vortex for 30 seconds.

21. 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.

22. Remove the supernatant carefully using pipette.

23. Repeat step 20-22 for a second wash.

24. Remove the 1.5 mL tube from the magnet, centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring all liquid to the bottom, place the 1.5 mL tube on magnet, until the solution clears and the nanoparticles are pelleted against the magnets.

25. Remove any liquid left in the bottom of 1.5 mL tube.

26. Keep the 1.5 mL tube on the magnet, air dry the nanoparticles for 3 minutes. (When environment humidity is high, time can be longer to minimize the residual amount of ethanol, which will affect elution efficiency.)

E. Elute cfDNA from magnetic nanoparticles

27. 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.

Plasma/serum volume	1 mL	2 mL	4 mL	8 mL
Suggested cfDNA Elution Solution Volume	20 uL	40 uL	80 uL	160 uL

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

29. 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.

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

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