Apostle MiniMax™ High Efficiency Total cfDNA/RNA (cfNAs) Isolation Kit Manual, 1mL x 50 Preps

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Product description

The Apostle MiniMax™ High Efficiency Total cfDNA/RNA (cfNAs) Isolation Kit is designed for isolation of DNA and RNA from cell free plasma and serum samples. The kit is featured for its efficient recovery of DNA in the range between 50-3000 bp, RNA, miRNA, and small RNA in the range between 17-1000 nt. The kit uses proprietary Apostle MiniMax™ technology, offers highly efficient recovery of high-quality cfDNA/RNA with high yield. The isolated DNA and RNA is suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

Kit capacity

The kit is capable of total cfDNA/RNA (cfNAs) isolation for 1 mL x 50 samples.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles	1.1 mL	2-8 °C
Binding Enhancer*	1.8 mL	-20 °C
Proteinase K	5 mL	Room Temperature, in dark
Total cfNAs Lysis/Binding Solution	55 mL	
Protein Precipitation Solution	3.3 mL	
Total cfNAs Wash Solution**	27.5 mL	
Total cfNAs Elution Solution	1.65 mL	

* Binding Enhancer shipped at ambient temperature. Immediately store it at -20 °C after receiving the kit. Thaw the solution before use.

** Before use, prepare Total cfNAs Wash Solution by adding 1 volume isopropanol to 1 volume Total cfNAs wash solution.

Total cfNAs Lysis/Binding Solution and Total cfNAs Wash Solution should be clear solution. If precipitate is observed in any of these reagents, warm the solution to 37 °C until the precipitate dissolves.

Read SDS before use. DO NOT ADD acids or bleach to any liquid wastes containing this product. Use ethanol if necessary.

Required materials not supplied

Adjustable micropipettes (1 mL, 200 uL, 20 uL) and tips
Magnets (specifically designed for 15 mL and 2 mL tubes)
Centrifuge (12000g, preferred), Table top centrifuge
Non-stick, low-binding, DNase/RNase-free tubes (1.5 mL and 15 mL)
Vortex, Shaker, Heater
Ethanol, 200 proof
Isopropanol, 100%
DNase/RNase-free water

Procedure for manual isolation of cfDNA/RNA

Note: For the preparation of plasma from whole blood, it is recommended to use ≤ 3000g for all centrifugation steps, to maximally preserve extracellular vesicles which contain cfDNA/RNA.

A. Sample treatment

1. Add components to a tube **in the order** indicated below, based on the volume of sample.

Reagents	Plasma/serum volume			
	500 uL	1 mL	2 mL	4 mL
Proteinase K	20 uL	40 uL	80 uL	160 uL
Plasma/serum	500 uL	1 mL	2 mL	4 mL
Total cfNAs Lysis/Binding Solution	50 uL	100 uL	200 uL	400 uL

Caution: avoid mixing proteinase K with cfDNA/RNA Lysis/Binding solution before Plasma/serum.

2. Vortex the solution well for 5 seconds, and incubate the mixture at 60 °C for 20 minutes.
3. At the end of the incubation, cool the tubes containing the plasma to room temperature.
4. Add Protein Precipitation Solution to the mixture, based on the sample volume indicated below. Vortex for 20 seconds, make sure the precipitation is uniformly dispersed. Incubate the mixture at room temperature for 3 minutes.

Plasma/serum volume	500 uL	1 mL	2 mL	4 mL
Protein Precipitation Solution	30 uL	60 uL	120 uL	240 uL

5. Centrifuge the mixture for 3 minutes at 12000g to pellet the precipitate. The supernatant should be clear.

Note: If centrifuge with 12000g capacity is not available, centrifugation can also be performed at 3000g for 10 minutes.

B. Bind cfDNA/RNA to magnetic nanoparticles

6. Transfer the supernatant from step 5 (~ 1 mL supernatant for each 1 mL initial plasma/serum) to a new 15 mL tube.
7. Add Binding Enhancer (**Brown Cap**) to the supernatant according to the table below, and mix well by vortexing for 5 s.

Plasma/serum volume	500 uL	1 mL	2 mL	4 mL
Binding Enhancer	16 uL	32 uL	64 uL	128 uL

8. Prepare the binding/nanoparticle solution in a new 15 mL tube according to the table below, and mix well. **Note**: Apostle MiniMax™ Magnetic Nanoparticles (**Green Cap**) should be brown solution. Equilibrate the vial to room temperature and vortex to fully resuspend the nanoparticles before use.

Reagents	Initial plasma/serum volume			
	500 uL	1 mL	2 mL	4 mL
cfDNA/RNA Lysis/Binding Solution	450 uL	900 uL	1.8 mL	3.6 mL
Magnetic Nanoparticles	10 uL	20 uL	30 uL	60 uL
Isopropanol (100%)	1 mL	2 mL	4 mL	8 mL

9. Add the prepared binding/nanoparticle solution to the mixture of binding enhancer and the supernatant in step 7. Thoroughly mix by vortexing briefly.

10. Shake at moderate-high speed for 10 minutes.

11. Place the tube on magnet for 3 min, or until the solution clears and the beads are pelleted against the magnet.

12. 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/RNA Wash Solution

13. Note: Prepare Wash solution by adding 1 volume of Isopropanol to 1 volume Apostle MiniMax™ cfDNA/RNA Wash Solution.

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

15. Carefully transfer the nanoparticle suspension to a new 1.5 mL tube, and save the lysis/binding tube. If necessary, briefly centrifuge the lysis/binding tube to bring all the solution to the bottom for easy transfer.

16. Place the new 1.5 mL tube on magnet for 1 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

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

18. Place the new 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/RNA from magnetic nanoparticles

27. Remove the 1.5 mL tube from the magnet, add Apostle MiniMax™ cfDNA/RNA Elution Solution (**Blue Cap**) to the 1.5 mL tube according to the following table, based on initial sample volume. RNase-free water can also be used as elution solution.

Plasma/serum volume	500 uL	1 mL	2 mL	4 mL
Suggested cfDNA/RNA Elution Solution Volume	20 uL	30 uL	40 uL	80 uL

28. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 minutes to elute the cfDNA/RNA 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/RNA in a non-stick, low-binding, DNase and RNase free microcentrifuge tube. Store the cfDNA/RNA sample at -80 °C.