Apostle MiniMax™ High Efficiency cfRNA Isolation Kit Manual, 200 uL x 50 Preps

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Product description

The Apostle MiniMax™ High Efficiency cfRNA Isolation Kit is designed for isolation of RNA from cell free plasma and serum samples. The kit is featured for its efficient recovery of 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 cfRNA with high yield. The isolated RNA is suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

Kit capacity

The kit is capable of cfRNA isolation for 200 uL x 50 samples.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles	550 uL	2-8°C
Binding Enhancer*	400 uL	-20 °C
Proteinase K	1 mL	Room Temperature, in dark
cfRNA Lysis/Binding Solution	11 mL	
Protein Precipitation Solution	660 uL	
cfRNA Wash Solution**	33 mL	
cfRNA 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 cfRNA Wash Solution by adding 5.5 volume isopropanol to 4.5 volume cfRNA wash solution.

All solutions stored at room temperature (15-30 °C) should be clear solution. If precipitate is observed in any reagent, warm the solution to 37 °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
Nonstick, DNase/RNase-free tubes (1.5 mL, 15 mL, 50 mL)
Vortex, Shaker, Heater
Ethanol, 200 proof
Isopropanol, 100%
Ultrapure DNase/RNase free water
DNase and DNase buffer (optional)

Procedure for manual isolation of cfRNA

Note: a. For the preparation of plasma from whole blood, it is recommended to use ≤ 3000g centrifugation force, to maximally preserve extracellular vesicles which contain cfRNA.

b. For qRT-PCR, cDNA synthesis method that includes a DNA removal step is recommended. If need to remove DNA during the extraction, perform on-bead DNase treatment in section F after step 26, before step 27.

A. Sample treatment

1. Add components to a 1.5 mL tube **in the order** indicated below, based on volume of sample.

Reagents	Plasma/serum volume			
	100 uL	200 uL	500 uL	1 mL
Proteinase K	4 uL	8 uL	20 uL	40 uL
Plasma/serum	100 uL	200 uL	500 uL	1 mL
cfRNA Lysis/Binding Solution	10 uL	20 uL	50 uL	100 uL

Caution: avoid mixing proteinase K with cfRNA 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 (**Yellow Cap**) 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	100 uL	200 uL	500 uL	1 mL
Protein Precipitation Solution	6 uL	12 uL	30 uL	60 uL

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

B. Bind cfRNA to magnetic nanoparticles

6. Transfer the supernatant from step 5 (~ 100 uL supernatant for each 100 uL initial plasma/serum) to a new tube (1.5 mL tube for 100-200 uL plasma, 15 ml tube for > 200 uL plasma).

7. Add binding enhancer (**Brown Cap**) to the supernatant according to the table below, and mix well by vortexing

for 5 s. Briefly centrifuge the tube to bring solution to the bottom.

Plasma/serum volume	100 uL	200 uL	500 uL	1 mL
Binding Enhancer	3.2 uL	6.5 uL	16 uL	32 uL

8. Prepare the binding/nanoparticle solution according to the table below, and mix well.
   Note: Apostle MiniMax™ Magnetic Nanoparticles (R) (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			
	100 uL	200 uL	500 uL	1 mL
cfRNA Lysis/Binding Solution	90 uL	180 uL	450 uL	900 uL
Magnetic Nanoparticles (R)	5 uL	10 uL	10 uL	20 uL
Isopropanol (100%)	250 uL	500 uL	1.25 mL	2.5 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. Briefly centrifuge the tube to bring solution to the bottom. 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™ cfRNA Wash Solution

13. Note: Prepare Wash solution by adding 5.5 volume of Isopropanol to 4.5 volume Apostle MiniMax™ cfRNA Wash Solution.

14. Remove the tube (referred to as lysis/binding tube below) from the magnet, add 250 uL of the prepared Apostle MiniMax™ cfRNA Wash Solution, vortex to resuspend the nanoparticles. Note: more prepared Apostle MiniMax™ cfRNA Wash Solution can be used to resuspend the nanoparticles in 15 mL lysis/binding tube.

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.)

*** If need to remove DNA from the final product during the extraction process, perform on-bead DNase treatment in section F after step 26, before step 27.**

E. Elute cfRNA from magnetic nanoparticles

27. Remove the 1.5 mL tube from the magnet, add Apostle MiniMax™ cfRNA 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	100 uL	200 uL	500 uL	1 mL
Suggested cfRNA Elution Solution Volume	15 uL	30 uL	30 uL	45 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 cfRNA 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 cfRNA in a non-stick, DNase and RNase free microcentrifuge tube. Store the cfRNA sample at -80 °C.

31. To characterize isolated cfRNA, DNase treatment is recommended. It is recommended to use PCR on chosen spike-in control or common housekeeping genes. Bioanalyzer 2100 with RNA 6000 Pico Kit or Small RNA kit can be also used to characterize the isolated cfRNA. Qubit measurement is not recomemded due to its low sensitivity and specificity. Nanodrop quantification should not be used due to its low sensitivity.

F. On-bead DNase treatment (Optional)

Perform section F only if DNA need to be removed during the extraction process, after step 26 in section D.

1. Remove the 1.5 mL tube from the magnet in step 26, add up to 50 uL DNase buffer containing DNase to the tube (e.g. 1 uL TURBO™ DNase mixed with 49 uL TURBO™ DNase buffer), and fully resuspend the nanoparticles by vortexing.

2. Incubate the solution for 15 mins at room temperature.

3. Add 1 ml of the prepared Apostle MiniMax™ cfRNA Wash Solution to the 1.5 mL tube, and shake at moderate-high speed for 10 minutes.

4. 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 1 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

5. Remove the supernatant carefully using pipette.

6. Remove the 1.5 mL tube from the magnet, add 1 mL 80% ethanol, then vortex for 30 seconds.

7. 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.

8. Remove the supernatant carefully using pipette.

9. Repeat step 6-8 in section F for a second wash.

10. 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.

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

12. 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.)

13. Continue with step 27 to elute cfRNA.