Apostle MiniMax® High Efficiency Cell-Free RNA Isolation Kit (1 mL × 50 preps), Instructions for Use

Manual isolation of cfRNA from plasma and serum

Catalog Number A18312-50 **Revision** Q.0

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 1 mL × 50 samples.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles	1.1 mL	2 to 30°C
Proteinase K	2.2 mL	
Binding Enhancer*	1.8 mL	-25 to -15°C
Lysis/Binding Solution	55 mL	15 to 30°C, in dark
Protein Precipitation Solution	3.3 mL	
Wash Solution**	33 mL	
Elution Solution	2.5 mL	

Note:

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

** Before each use, prepare **cfRNA wash mixture** by adding 5.5 volume of isopropanol to 4.5 volume of Wash Solution, and mix well.

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

Required materials not supplied

- Adjustable micropipettes and tips (20, 200, and 1000 µL)
- Magnetic rack (designed for 15 mL and 2 mL tubes)
- Centrifuge (12,000×g), Table top centrifuge
- Nonstick, nuclease-free tubes (1.5 mL, 15 mL, 50 mL)
- Vortex
- Thermal shaker or incubator (for sample lysis)
- Ethanol, 200 proof, molecular biology grade
- Isopropanol, 100%
- Water, nuclease-free
- DNase and DNase buffer (optional)

Procedure for manual isolation of cfRNA

Note:

- For the preparation of plasma from whole blood, it is recommended to use ≤ 3000×g centrifugation force, to maximally preserve extracellular vesicles which contain cfRNA.
- For qRT-PCR, cDNA synthesis method that includes a DNA removal step is recommended. If needed to remove DNA during the extraction, perform on-bead DNase treatment in section E, step 27 to 38.

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 µL	200 µL	500 µL	1 mL
Proteinase K	4 µL	8 µL	20 µL	40 µL
Plasma/serum	100 µL	200 µL	500 µL	1 mL
Lysis/Binding Solution	10 µL	20 µL	50 µL	100 µL

Caution: avoid mixing proteinase K with Lysis/Binding Solution before plasma/serum.

2. Vortex the solution well for 5 seconds, and incubate the mixture at 60°C for 20 mins.
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 mins.

Initial plasma/serum volume	100 µL	200 µL	500 µL	1 mL
Protein Precipitation Solution	6 µL	12 µL	30 µL	60 µL

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

Note: If centrifuge with 12,000×g capacity is not available, centrifugation can also be performed at 3,000×g for 10 mins.

B. Bind cfRNA to magnetic nanoparticles

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

Initial plasma/serum volume	100 µL	200 µL	500 µL	1 mL
Binding Enhancer	3.2 µL	6.5 µL	16 µL	32 µL

8. Prepare the binding mixture according to the table below, and mix well.

Note: Apostle MiniMax® Magnetic Nanoparticles (**Green Cap**) should be a brown solution. Equilibrate the vial to room temperature and vortex to fully resuspend the nanoparticles before use.

Reagents	Initial plasma/serum volume			
	100 µL	200 µL	500 µL	1 mL
Lysis/Binding Solution	90 µL	180 µL	450 µL	900 µL
Magnetic Nanoparticles	5 µL	10 µL	10 µL	20 µL
Isopropanol (100%)	250 µL	500 µL	1.25 mL	2.5 mL

9. Add the prepared binding mixture 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 mins.
11. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on the magnetic rack for 3 mins, 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 cfRNA wash mixture

13. Prepare cfRNA wash mixture by adding 5.5 volume of Isopropanol to 4.5 volume Apostle MiniMax® cfRNA Wash Solution, and mix well.
14. Remove the tube (referred to as lysis/binding tube below) from the magnetic rack, add 250 µL of the prepared cfRNA wash mixture, vortex to resuspend the nanoparticles.

Note: more prepared cfRNA wash mixture can be used to resuspend the nanoparticles in a 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 the magnetic rack 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 the magnetic rack for 2 mins, or until the solution clears and the nanoparticles are pelleted against the magnets.
19. Remove the supernatant carefully using a pipette.

D. Second Wash with 80% Ethanol

20. Remove the 1.5 mL tube from the magnetic rack, 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 the magnetic rack for 2 mins, or until the solution clears and the nanoparticles are pelleted against the magnets.
22. Remove the supernatant carefully using a pipette.
23. Repeat step 20-22 for a second wash.
24. Remove the 1.5 mL tube from the magnetic rack, centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring all liquid to the bottom, place the 1.5 mL tube on the magnetic rack, until the solution clears and the nanoparticles are pelleted against the magnets.
25. Remove any liquid left in the bottom of the 1.5 mL tube.
26. Keep the 1.5 mL tube on the magnetic rack, air dry the nanoparticles for 3 mins. (When environment humidity is high, time can be longer to minimize the residual amount of ethanol, which will affect elution efficiency.)

E. On-bead DNase treatment (Optional)

*** If DNA need to be removed from the final product during the extraction process, perform on-bead DNase treatment in this section. Otherwise skip to step 39.**

27. Remove the 1.5 mL tube from the magnet in step 26, add up to 50 µL DNase buffer containing DNase to the tube (e.g. 1 µL TURBO™ DNase mixed with 49 µL TURBO™ DNase buffer), and fully resuspend the nanoparticles by vortexing.
28. Incubate the solution for 15 mins at room temperature.
29. Add 1 ml of the prepared cfRNA wash mixture (adding 5.5 volume of Isopropanol to 4.5 volume Apostle MiniMax® cfRNA Wash Solution) to the 1.5 mL tube, and shake at moderate-high speed for 10 mins.

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 magnetic rack for 1 min, or until the solution clears and the nanoparticles are pelleted against the magnets.
31. Remove the supernatant carefully using a pipette.
32. Remove the 1.5 mL tube from the magnetic rack, add 1 mL 80% ethanol, then vortex for 30 seconds.
33. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on the magnetic rack for 2 mins, or until the solution clears and the nanoparticles are pelleted against the magnets.
34. Remove the supernatant carefully using a pipette.
35. Repeat step 32-34 in section F for a second wash.
36. Remove the 1.5 mL tube from the magnetic rack, centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring all liquid to the bottom, place the 1.5 mL tube on the magnetic rack, until the solution clears and the nanoparticles are pelleted against the magnets.
37. Remove any liquid left in the bottom of the 1.5 mL tube.
38. Keep the 1.5 mL tube on the magnetic rack, air dry the nanoparticles for 3 mins. (When environment humidity is high, time can be longer to minimize the residual amount of ethanol, which will affect elution efficiency.)

F. Elute cfRNA from magnetic nanoparticles

39. Remove the 1.5 mL tube from the magnetic rack, add Elution Solution to the 1.5 mL tube according to the following table, based on initial sample volume. RNase-free water can also be used as an elution solution.

Initial plasma/serum volume	100 µL	200 µL	500 µL	1 mL
Recommended Elution Solution Volume	15 µL	30 µL	30 µL	45 µL

40. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 mins to elute the cfRNA from the nanoparticle.
41. Centrifuge the 1.5 mL tube using tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on the magnetic rack, until the solution clears and the nanoparticles are pelleted against the magnets.
42. Collect the supernatant that contains cfRNA in a non-stick, DNase/RNase-free microcentrifuge tube. Store the cfRNA sample at -80°C.
43. 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 recommended due to its low sensitivity and specificity. Nanodrop quantification should not be used due to its low sensitivity.