

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



Manual isolation of cfDNA/RNA from plasma and serum

Catalog Number A18725-50 Revision Q.1

Product description

The Apostle MiniMax[®] High Efficiency 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 cfNAs isolation for 1 mL × 50 samples.

Kit contents and storage condition

Contents	Amount	Storage
Box 1 of 2		
Magnetic Nanoparticles	1.1 mL	2 to 30°C
Proteinase K	5 mL	
Lysis/Binding Solution	55 mL	15 to 30°C, in dark
Protein Precipitation Solution	3.3 mL	
Wash Solution**	27.5 mL	
Elution Solution	1.65 mL	
Box 2 of 2		
Binding Enhancer*	1.8 mL	-25 to -15°C

Note:

* **Binding Enhancer (in Box 2 of 2)** are shipped at ambient temperature. Immediately store it at -20°C after receiving and thaw the solution before use.

** Before each use, prepare **cfNAs wash mixture** by adding 1 volume of isopropanol to 1 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

Procedure for manual isolation of cfNAs

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 cfDNA/RNA.

A. Sample treatment

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

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

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

- Vortex the solution well for 5 seconds, and incubate the mixture at 60°C for 20 mins.
- At the end of the incubation, cool the tubes containing the plasma to room temperature.
- 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	500 µL	1 mL	2 mL	4 mL
Protein Precipitation Solution	30 µL	60 µL	120 µL	240 µL

- 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 cfNAs to magnetic nanoparticles

- Transfer the supernatant from step 5 (~1 mL supernatant for each 1 mL initial plasma/serum) to a new 15 mL tube.

- Add binding enhancer (**Brown Cap**) to the supernatant according to the table below, and mix well by vortexing for 5 seconds.

Initial plasma/serum volume	500 μ L	1 mL	2 mL	4 mL
Binding Enhancer	16 μ L	32 μ L	64 μ L	128 μ L

- Prepare the binding mixture in a new 15 mL tube 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			
	500 μ L	1 mL	2 mL	4 mL
Lysis/Binding Solution	450 μ L	900 μ L	1.8 mL	3.6 mL
Magnetic Nanoparticles	10 μ L	20 μ L	30 μ L	60 μ L
Isopropanol (100%)	1 mL	2 mL	4 mL	8 mL

- Add the prepared binding mixture to the mixture of binding enhancer and the supernatant in step 7. Thoroughly mix by vortexing briefly.
- Shake at moderate-high speed for 10 mins.
- Place the tube on the magnetic rack for 3 mins, or until the solution clears and the beads are pelleted against the magnet.
- 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 cfNAs wash mixture

- Prepare cfNAs wash mixture by adding 1 volume of Isopropanol to 1 volume Apostle MiniMax[®] cfNAs Wash Solution and mix well.
- Remove the tube (referred to as lysis/binding tube below) from the magnetic rack, add 1 mL of the prepared cfNAs wash mixture, vortex to resuspend the nanoparticles.
- 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.
- Place the new 1.5 mL tube on a magnetic rack for 1 min, or until the solution clears and the nanoparticles are pelleted against the magnets.

- 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.

- 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.

- Remove the supernatant carefully using a pipette.

D. Second Wash with 80% Ethanol

- 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.

- 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.

- Remove the supernatant carefully using a pipette.

- Repeat step 20-22 for a second wash.

- 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.

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

- 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. Elute cfNAs from magnetic nanoparticles

- Remove the 1.5 mL tube from the magnetic rack, add 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 an elution solution.

Initial plasma/serum volume	500 μ L	1 mL	2 mL	4 mL
Recommended Elution Solution Volume	20 μ L	30 μ L	40 μ L	80 μ L

- Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 mins to elute the cfNAs from the nanoparticle.

- 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.

- Collect the supernatant that contains cfNAs in a non-stick, DNase/RNase-free microcentrifuge tube. Store the cfRNA sample at -80°C.