# Apostle MiniMax® High Efficiency Cell-Free DNA Isolation Kit (5 mL × 50 preps), Instructions for Use

Manual isolation of cfDNA from plasma, serum, and urine samples

**Catalog Number** A17622-250 **Revision** Q.0

## Product description

The Apostle MiniMax® High Efficiency cfDNA Isolation Kit is designed for isolation of DNA from cell free plasma, serum, or urine samples. The kit uses proprietary Apostle MiniMax® technology, and offers highly efficient, reproducible recovery of high-quality cfDNA with high yield. The isolated DNA samples are suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

## Kit capacity

The kit is capable of cfDNA isolation from 50 samples of 5 mL each.

## Kit contents and storage

| Component | Amount | Storage |
|---|---|---|
| Magnetic Nanoparticles | 4.2 mL | 2 to 30°C |
| Proteinase K | 11 mL | |
| Sample Lysis Buffer | 27.5 mL | 15 to 30°C, in dark |
| Lysis Binding Solution | 345 mL | |
| Wash Solution | 110 mL | |
| 2nd Wash Solution | 27.5 mL | 15 to 30°C |
| Elution Buffer | 8.5 mL | |

**Note:** Magnetic Nanoparticle solution should be a brown suspension. Vortex the magnetic nanoparticle solution to fully resuspend the nanoparticles right before use.

Sample Lysis Buffer, Lysis/Binding Solution, and Wash Solution should be clear to light yellow in color. If a 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

- Ethanol, 200 proof
- Adjustable micropipettes and tips (20, 200, and 1000 µL)
- Nuclease-free/low-binding tubes (1.5 and 15 mL)
- Magnetic tube racks (1.5 and 15 mL)
- Vortex or shaker
- Water bath or heater
- Tabletop centrifuge

## Manual isolation procedure

### A. Sample lysis

1. Add components to a 15 mL tube **in the order** indicated below, based on volume of sample.

| Reagent | Volume | | | Unit |
|---|---|---|---|---|
| Proteinase K | 40 | 80 | 200 | µL |
| Sample | 1 | 2 | 5 | mL |
| Sample Lysis Buffer | 100 | 200 | 500 | µL |

**Caution:** avoid mixing Proteinase K with Sample Lysis Buffer before adding to the mixture.

2. Mix the solution well by vortexing briefly, then incubate the mixture at 60°C for 20 minutes.
3. At the end of the incubation, cool the tubes containing the samples to room temperature.

### B. Bind cfDNA to Nanoparticles

4. For each sample, prepare the binding/nanoparticle solution according to the table below and mix well (**Note:** equilibrate the Magnetic Nanoparticles (**green cap**) to room temperature and then vortex to fully resuspend the nanoparticles right before use):

| Reagent | Initial sample volume | | | Unit |
|---|---|---|---|---|
| | 1 | 2 | 5 | mL |
| Lysis/Binding Solution | 1.25 | 2.5 | 6.25 | mL |
| Magnetic Nanoparticles | 15 | 30 | 75 | µL |

5. Add the prepared binding/nanoparticle solution to the sample, thoroughly mix by vortexing briefly, or invert the tube 10 times (**Note:** avoid excessive vortexing, which generates excessive bubbles).
6. Shake at moderate-high speed for 10 minutes to bind the cfDNA to the nanoparticles, e.g., isotherm shaker at 1,200 to 2,200 rpm.
7. Place the tube on a magnet for 5 minutes, or until the solution clears and the beads are pelleted against the magnet.
8. Carefully remove and discard the supernatant with a pipette.

**C. Wash with cfDNA Wash Solution**

9. Remove the tube (referred to as lysis/binding tube below) from the magnet, add 1 mL of cfDNA Wash Solution, then vortex to resuspend the nanoparticles.
10. Transfer the magnetic nanoparticle suspension to a new low-bind 1.5 mL microcentrifuge tube and save the lysis/binding tube.
11. Place the 1.5 mL tube on the magnet to pellet the nanoparticles for 1 min.
12. Use the supernatant in the 1.5 mL tube to rinse the saved lysis/binding tube and transfer any residual nanoparticles to the 1.5mL tube, then discard the lysis/binding tube.
13. Place the 1.5 mL tube on the magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnet.
14. Remove and discard the supernatant carefully with a pipette.
15. Remove the 1.5 mL tube from the magnet, add 1 mL of cfDNA Wash Solution, then vortex for 30 seconds.
16. Briefly centrifuge the 1.5 mL tube using tabletop centrifuge to bring solution to the bottom, then place the 1.5 mL tube on magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnet.
17. Remove and discard the supernatant carefully using a pipette.

**D. Wash with cfDNA 2nd Wash Solution**

18. For each sample, prepare the washing mixture with 0.4 mL of cfDNA 2nd Wash Solution and 1.6 mL of ethanol, 200 proof.
19. Remove the 1.5 mL tube from the magnet, add 1 mL of the washing mixture, then vortex for 30 seconds.
20. Briefly centrifuge the 1.5 mL tube using a tabletop centrifuge to bring solution to the bottom, place the 1.5 mL tube on the magnet for 2 min, or until the solution clears and the nanoparticles are pelleted against the magnet.
21. Remove and discard the supernatant carefully using a pipette.
22. Repeat steps 19-21 for a second wash.
23. Remove the 1.5 mL tube from the magnet, briefly centrifuge the 1.5 mL tube using a tabletop centrifuge to bring all liquid to the bottom, and place the 1.5 mL tube on magnet, until the solution clears and the nanoparticles are pelleted against the magnet
24. Remove and discard any liquid left in the bottom of the 1.5 mL tube.
25. Keeping the 1.5 mL tube on the magnet, air dry the nanoparticles for 3 minutes. (When environmental humidity is high, drying time should be longer to minimize the residual amount of ethanol, which will affect elution efficiency.)

**E. Elute cfDNA from Nanoparticles**

26. Remove the 1.5 mL tube from the magnet, add cfDNA Elution Solution (**blue cap**) to the 1.5 mL tube according to the following table, based on initial sample volume.

| **Initial sample volume** | **1 mL** | **2 mL** | **5 mL** |
|---|---|---|---|
| Suggested elution volume | 20 µL | 40 µL | 100 µL |

27. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 minutes to elute the cfDNA from the nanoparticle.
28. Briefly centrifuge the 1.5 mL tube using a tabletop centrifuge to bring solution to the bottom, then place the 1.5 mL tube on a magnet, until the solution clears and the nanoparticles are pelleted against the magnet.
29. Collect the supernatant containing cfDNA in a nuclease-free/low-binding microcentrifuge tube.
30. Store the cfDNA eluate at 4°C for short term storage, and -20°C for long term storage.
31. If characterization and quantification of the isolated cfDNA eluate is needed, it is recommended to use Agilent Bioanalyzer 2100+ High Sensitivity DNA Analysis Kit (Cat# 5067-4626), due to its low detection limit (5 pg/µL).