Apostle MiniGenomics® Whole Blood Genomic DNA Isolation Kit (200 µL by 200 preps), Instructions for Use

Manual isolation of genomic DNA from whole blood sample

Catalog Number A230814-200 **Revision** A.0

Product description

The Apostle MiniGenomics® Whole Blood Genomic DNA Isolation Kit is designed for rapid isolation of high-quality, ready-to-use genomic DNA from fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants. The kit uses proprietary Apostle MiniGenomics® technology, and offers highly efficient, reproducible recovery of high-quality DNA with high yield. This kit is designed for input volume range from 100 µL to 350 µL. The protocol can be performed in both 96-well plate (manually and automated) and single tube formats (manually only). The isolated DNA samples are suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

Kit capacity

The kit is capable of gDNA isolation for 40 mL human whole blood.

Kit contents and storage condition

Contents	Amount	Storage
Magnetic Nanoparticles	660 µL	2 to 30°C
Proteinase K	4.4 mL	
Lysis/Binding Solution	22 mL	15 to 30°C, in dark
Lysis Enhancer	2.7 mL	
Wash Solution	880 mL	
Elution Solution	44 mL	
Binding Enhancer*	530 µL	-25 to -15°C

Note: Magnetic nanoparticle solution should be brown solution. Vortex magnetic nanoparticle solution to fully resuspend the nanoparticles before use.

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

Lysis/Binding Solution and Wash Solution should be clear solutions. 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 µL, 20 µL, 10 µL) and tips
- Magnets (specifically designed for 15 mL and 2 mL tubes)
- Tabletop centrifuge
- DNase/RNase-free tubes (1.5 mL and 15 mL)
- Vortex
- Shaker
- Ethanol, 200 proof, Molecular Biology Grade
- 80% Ethanol, Molecular Biology Grade
- Heater (for sample lysis)

Procedure for manual isolation of gDNA

A. Sample lysis

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

Reagents	Whole blood volume		
	100 µL	200 µL	350 µL
Proteinase K	10 µL	20 µL	35 µL
Whole blood	100 µL	200 µL	350 µL
Lysis Enhancer	6 µL	12 µL	20 µL
Lysis/Binding Solution	50 µL	100 µL	175 µL

Caution: avoid mixing proteinase K, Lysis/Binding Solution, and Lysis Enhancer (**yellow cap**) before blood sample.

2. Mix the solution well by vortexing briefly and incubate the mixture at 60°C for 10 minutes.
3. At the end of the incubation, cool the tubes containing the blood sample to room temperature.

B. Bind gDNA to magnetic nanoparticles

4. Prepare the binding/nanoparticle mixture according to the table below, and mix well (**Note**: preincubate the Apostle MiniMax® Magnetic Nanoparticles vial (**Green Cap**) to room temperature and then vortex to fully resuspend the nanoparticles before use):

Reagents	Whole blood volume		
	100 µL	200 µL	350 µL
Ethanol	110 µL	220 µL	380 µL
Magnetic Nanoparticles	1.5 µL	3 µL	5 µL
Binding Enhancer	1.2 µL	2.4 µL	4 µL

5. Add the prepared binding/nanoparticle mixture to the lysate, thoroughly mix by vortexing.
6. Shake at moderate-high speed for 5 minutes to bind the gDNA to the nanoparticles.
7. Centrifuge the 1.5 mL tube using a tabletop centrifuge briefly to bring solution to the bottom, place the 1.5 mL tube on magnet for 2 minutes, or until all the nanoparticles are pelleted against the magnets.
8. Carefully remove the supernatant with pipette.

C. Wash with Wash Solution

9. Remove the tube from the magnet, add 1 mL of Wash Solution, vortex to resuspend the nanoparticles (**Note**: time of vortex mix can be appropriately extended for beads to be fully dispersible in solution. In some cases, some of the resuspended beads may form clumps with no significant effect on gDNA purity).
10. Centrifuge the 1.5 mL tube using a 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.
11. Remove the supernatant carefully using a pipette (**Note**: ensure that the Wash steps are performed correctly.
12. Repeat step 9-11 for a second wash to improve the purity if needed.

D. Wash with 80% Ethanol

13. Remove the 1.5 mL tube from the magnet, add 1 mL of 80% ethanol, then vortex for 20 seconds.
14. 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 minute, or until the solution clears and the nanoparticles are pelleted against the magnets.
15. Remove the supernatant carefully using pipette.
16. Repeat steps 13-15 for a second wash.
17. 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 all the nanoparticles are pelleted against the magnets.
18. Remove any liquid left in the bottom of 1.5 mL tube.
19. 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 gDNA from magnetic nanoparticles

20. Remove the 1.5 mL tube from the magnet, add Elution Solution to the 1.5 mL tube according to the following table, based on initial sample volume.

Whole blood volume	100 µL	200 µL	350 µL
Elution Solution volume	30 µL	60 µL	100 µL

21. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, incubate at 60°C for 5 minutes and shake at 2,200 rpm for 3 minutes to elute the gDNA from the nanoparticle.
22. 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.
23. Collect the supernatant that contains gDNA in a DNase and RNase free microcentrifuge tube.
24. Store the gDNA sample at 4°C for short term storage, or -20°C or -80°C for long term storage.