Apostle MiniGenomics® Multi-Sample Fast Kit (50 Preps), Instructions for Use

Manual isolation of genomic DNA from tissue/cultured cell sample

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Product description

The Apostle MiniGenomics® Multi-Sample Fast Kit is designed for rapid isolation of high-quality, ready-to-use genomic DNA from saliva, buccal swab, tissue/cultured cell, and peripheral blood buffy coat. The kit uses proprietary Apostle MiniGenomics® technology, offers highly efficient, reproducible recovery of high-quality DNA with high yield. 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 500 mg human tissue or 2.5×10^7 cells.

Kit contents and storage condition

Component	Amount	Storage
Magnetic Nanoparticles	1.1 mL	2 to 30°C
Proteinase K	2.2 mL	
Lysis/Binding Solution	11 mL	15 to 30°C, in the dark
Wash Solution	28 mL	
2nd Wash Solution	22 mL	
Elution Solution	11 mL	

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

Lysis/Binding Solution and Wash Solution should be clear solution. 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, and 20 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
- PBS, sterile
- Heater (for sample lysis)
- Surgical blade/scissor with glass dish or grinding beads

Procedure for manual isolation of gDNA

A. Sample preparation

For tissue sample

1. Take 5 to 10 mg of fresh/frozen tissue samples and place them in a clean glass dish. Use a disposable surgical blade or surgical scissor to chop/cut them into small pieces then transfer the chopped tissue into a 1.5 mL centrifuge tube.

   Alternatively, add 5 to 10 mg of fresh/frozen tissue directly into a 1.5 mL centrifuge tube and add an appropriate amount of grinding beads. Vortex the tube until the tissue is ground into a homogenized mixture.

2. Top up the prepared tissue/cellular homogenate with 1× PBS to a final volume of 200 µL

For cultured cell sample

1. Take up to 5×10^5 cells in pellet form and thaw the cell pellets at room temperature if needed. Remove as much media as possible from cell pellets and transfer the cell pellets into a 1.5 mL centrifuge tube.
2. Top up the cells with 1× PBS to a final volume of 200 µL, then vortex to dislodge the cells from the tube wall until the cells form a clear, viscous, and free-floating mass.

B. Sample lysis

3. Add 40 µL Proteinase K and 200 µL Lysis/Binding Solution to the 1.5 mL tube with sample (**Note:** avoid premix of Proteinase K and Lysis/Binding Solution to affect lysis).
4. Mix the solution well by vortexing briefly and incubate the mixture at 60°C for 1 hours or above (overnight is applicable). Invert the mixture 2-3 times every hour until the mixture is fully dissolved.
5. At the end of the incubation, cool the tubes containing the sample to room temperature.

C. Bind gDNA to magnetic nanoparticles

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

Reagents	Volume per prep
Ethanol, 200 proof	300 µL
Magnetic Nanoparticles	20 µL

7. Add the prepared binding/nanoparticle solution to the sample tube, thoroughly mix by vortexing briefly (**Note**: avoid excessive vortexing, which generate excessive bubbles).
8. Shake at moderate-high speed for 5 minutes to bind the gDNA to the nanoparticles.
9. 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 minutes, or until the solution clears and the nanoparticles are pelleted against the magnets.
10. Carefully remove and discard the supernatant with pipette.

D. Wash with Wash Solution

11. Remove the tube from the magnet, add 500 µL 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 rare cases, some of the resuspended beads may form clumps with no significant effect on gDNA purity).
12. 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.
13. Remove and discard the supernatant carefully using pipette (**Note**: ensure that the Wash steps are performed correctly. Consider repeating step 11-13 for a second wash to improve the purity if needed).

E. Wash with 2nd Wash Solution

14. Pre-dilute 2nd Wash Solution 1:4 in Ethanol, 200 proof before use, to a final composition of 20% 2nd Wash Solution and 80% Ethanol. The amount of final secondary wash buffer required is 2 mL per sample.
15. Remove the 1.5 mL tube from the magnet, add 1 mL of the prepared secondary wash buffer (20% 2nd Wash Solution, with 80% Ethanol), then vortex for 20 seconds.
16. 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.
17. Remove the supernatant carefully using pipette.
18. Repeat step 18-20 for a second wash.
19. 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.
20. Remove any liquid left in the bottom of 1.5 mL tube.
21. 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)

F. Elute gDNA from magnetic nanoparticles

22. Remove the 1.5 mL tube from the magnet, add 50 to 200 µL Elution Solution to the 1.5 mL tube.
23. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 3 minutes to elute the gDNA from the nanoparticle.
24. 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.
25. Collect the supernatant that contains gDNA in a DNase and RNase free microcentrifuge tube.
26. Store the gDNA sample at 4°C for short term storage, and -20°C or -80°C for long term storage.