

Apostle MiniGenomics® Stool DNA Isolation Kit (50 Preps), Instructions for Use



Manual isolation of genomic DNA from stool sample

Catalog Number A181206-50 Revision A.3

Product description

The Apostle MiniGenomics® Stool DNA Isolation 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 50 preps of fresh, frozen stool (180~220 mg/prep recommended) or preserved stool homogenate (200 µL/prep).

Kit contents and storage condition

Component	Amount	Storage
Magnetic Nanoparticles	1.1 mL	2 to 30°C
Proteinase K	2.2 mL	
Sample Lysis Buffer A	1.4 mL	15 to 30°C, in the dark
Sample Lysis Buffer B	2.8 mL	
Lysis/Binding Solution	11 mL	
Wash Solution	13.5 mL	
Elution Solution	11 mL	

Note:

- Before first use, add **16.5 mL Ethanol, 200 proof** into **Wash Solution** and mix well. Check the checkbox on label to record the completion of ethanol addition.
- Before each use, prepare 80% ethanol with ethanol, 200 proof, molecular biology grade and nuclease-free water. 80% ethanol needed for each sample is 2 mL.
- Magnetic nanoparticle solution should be brown solution. Vortex magnetic nanoparticle solution to fully resuspend the nanoparticles before use.
- Sample Lysis Buffer A/B, 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
- Magnetic stand (specifically 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
- PBS, sterile
- Heater (for sample lysis)

Procedure for manual isolation of gDNA

A. Sample lysis

1. For each solid stool sample, transfer appropriate amount (according to the following table) of fresh/frozen stool samples and PBS into a 1.5 mL tube.

Solid stool sample	PBS volume
80~120 mg	100 µL
180~220 mg	200 µL
380~420 mg	400 µL

For each liquid stool sample or sample stored in preservation solution, vortex the original stool samples for homogenization, and transfer 100 to 400 µL fecal homogenate into a 1.5 mL microcentrifuge tube.

2. Add Sample Lysis Buffer A into the stool sample and mix well, then add Sample Lysis Buffer B and mix well (according to the following table).

Reagent	Volume per prep (µL)		
Solid stool in PBS/stool homogenate	100	200	400
Sample Lysis Buffer A	12.5	25	50
Sample Lysis Buffer B	25	50	100

Caution: avoid mixing Sample Lysis Buffer A with Sample Lysis Buffer B before adding to the sample.

3. Vortex continuously for 5 minutes or until the stool sample is thoroughly homogenized and lysed.

Note: it is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

4. Centrifuge the sample at 13,000 × g for 5 minutes to pellet residue stool particles.

- Add Proteinase K to a new 1.5 mL microcentrifuge tube, then transfer the supernatant contains stool sample lysate to the tube and add appropriate amount of Lysis/Binding Solution (according to the following table).

Reagent	Initial sample volume		
	100 μ L	200 μ L	400 μ L
Proteinase K	20 μ L	40 μ L	80 μ L
Stool sample lysate	100 μ L	200 μ L	400 μ L
Lysis/Binding Solution	100 μ L	200 μ L	400 μ L

Caution: avoid mixing proteinase K with Lysis/Binding Solution before adding to the sample lysate. Do not transfer any solid material in the sample lysate. If particles are still visible in the supernatant, centrifuge the sample lysate again.

- Mix the solution well by vortexing briefly and incubate the mixture at 70°C for 10 minutes.
- At the end of the incubation, cool the tube with mixture to room temperature.

B. Bind gDNA to magnetic nanoparticles

- Prepare the binding/nanoparticle solution according to the table below and mix well.

Reagents	Volume per prep, for every 100 μ L of sample lysate
Ethanol, 200 proof	150 μ L
Magnetic Nanoparticles	10 μ L

Note: equilibrate the MiniGenomics® Magnetic Nanoparticles vial (**Green Cap**) to room temperature and then vortex to fully resuspend the nanoparticles before use. The reagent volume can be scaled up or down according to sample lysate volume.

- Add the prepared binding/nanoparticle solution to the sample, thoroughly mix by vortexing briefly, or invert the tube 10 times.

Note: avoid excessive vortex, which generate excessive bubbles.

- Shake at moderate-high speed for 5 minutes to bind the gDNA to the nanoparticles.
- 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 stand for 2 minutes, or until the solution clears and the nanoparticles are pelleted against the magnets.
- Carefully remove the supernatant with pipette.

C. Wash with Wash Solution (premixed with ethanol)

- Remove the tube from the magnetic stand, add 500 μ L of Wash Solution (premixed with ethanol), vortex to resuspend the nanoparticles

Note: check the label of the Wash Solution to ensure that ethanol has been added before the first use. Time of vortex mixing can be extended for the beads to be fully dispersed in

the solution. In rare cases, some of the resuspended beads may form clumps with no significant effect on gDNA purity.

- 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 stand for 1 minute, or until the solution clears and the nanoparticles are pelleted against the magnets.

- Remove the supernatant carefully using pipette.

Note: Consider repeating step 12-14 for a second wash to improve the purity if needed.

D. Wash with 80% ethanol

- Remove the 1.5 mL tube from the magnetic stand, add 1 mL of the prepared 80% ethanol, then vortex for 20 seconds.

Note: time of vortex mixing can be extended for beads to be fully dispersed in solution.

- 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 stand for 1 minute, or until the solution clears and the nanoparticles are pelleted against the magnets.

- Remove the supernatant carefully using pipette.

- Repeat step 16-18 for a second wash.

- 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 the magnetic stand, until the solution clears and the nanoparticles are pelleted against the magnets.

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

- Keep the 1.5 mL tube on the magnetic stand, air dry the nanoparticles for 3 minutes.

Note: when environment humidity is high, time can be extended to minimize the liquid residual, which will affect elution efficiency.

E. Elute gDNA from magnetic nanoparticles

- Remove the 1.5 mL tube from the magnetic stand, add 50~100 μ L Elution Solution to the 1.5 mL tube.

- 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.

- 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 stand, until the solution clears and the nanoparticles are pelleted against the magnets.

- Collect the supernatant that contains gDNA in a DNase and RNase free microcentrifuge tube.

- Store the gDNA sample at 4°C for short term storage, and -20°C or -80°C for long term storage.