# Apostle MiniGenomics® Total RNA Isolation Kit (100 µL×200 preps), Instructions for Use

Manual isolation of total RNA from blood, cultured cells, or tissue

**Catalog Number** A220412-200 **Revision** A.0

## Product description

The Apostle MiniGenomics® Total RNA Isolation Kit is designed for rapid, simple isolation of high-quality, ready-to-use total RNA from blood, cultured cells, or tissue samples. The kit uses proprietary Apostle MiniGenomics® technology, offering highly efficient, reproducible recovery of high-quality RNA with high yield. The protocol allows processing in either 96-well plates (manual or automated) or single tubes (manual). The isolated RNA are suitable for a broad range of subsequent applications, including sequencing, PCR, etc.

## Kit capacity

The kit is capable of RNA isolation for 50 preps of blood samples (100 µL blood each sample), cell suspension samples (cell number of each 100 µL sample is $\leq 1\times10^6$), or small amount of tissue samples (≤30 mg).

## Kit contents and storage condition

| Content | Amount | Storage |
|---|---|---|
| Magnetic Nanoparticles (**Green Cap**) | 2.2 mL | 2 to 30°C |
| Proteinase K | 8.2 mL | |
| DNase Buffer | 22 mL | |
| DNase | 220 µL | -25 to -15°C |
| Binding Enhancer (**Brown Cap**) | 800 µL | |
| Lysis/Binding Solution | 16.8 mL | 15 to 30°C, in dark |
| Re-binding Solution | 17.2 mL | |
| Wash Solution | 86.4 mL | |
| RNA Elution Solution | 22 mL | |

**Note:**

- **DNase** and **Binding Enhancer** are shipped with dry ice. Immediately store them at -20°C after receiving and thaw them before use.
- Before first use, add **32 mL isopropanol** into **Re-binding Solution** and mix well. Check the checkbox on label to record the completion of IPA addition.
- Before first use, add **105.6 mL isopropanol** into **Wash Solution** and mix well. Check the checkbox on label to record the completion of IPA 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 1.2 mL.
- All solutions stored at room temperature (15 to 30°C) should be clear. If precipitate is observed in any of these reagents, warm the solution to 37°C until the precipitate dissolves.
- Magnetic nanoparticle solution should be brown solution. Vortex magnetic nanoparticle solution to fully resuspend the nanoparticles before use.

## Required materials not supplied

- Adjustable micropipettes and tips (20, 200, and 1000 µL)
- Magnetic rack (specifically designed for 15 mL and 2 mL tubes)
- Tabletop centrifuge
- Nonstick, DNase/Rnase-free tubes (1.5 mL)
- Vortex
- Thermal shaker or incubator (for sample lysis)
- β-mercaptoethanol (BME)
- Ethanol, 200 proof, molecular biology grade
- Isopropanol, 100%
- Water, nuclease-free
- PBS, nuclease-free (optional)

## Sample requirements

Samples: Blood samples (Collected by anticoagulant blood collection tube. Do not freeze); Cultured cells samples (Trypsinized and suspended in nuclease-free PBS. Cell number of each 100 µL sample is $\leq 1\times10^6$); Tissue samples (≤30 mg of tissue sample homogenized with 200 µL nuclease-free PBS).

Sample storage: Process the sample immediately after collection, or temporarily stored at 4°C. Do not freeze.

## Procedure for manual isolation of total RNA

### A. Sample lysis and nucleic acid binding

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

| Reagents | Blood/Cell suspension volume | | |
|---|---|---|---|
| | 50 µL | 100 µL | 200 µL |
| Lysis/Binding Solution | 37.5 µL | 75 µL | 150 µL |
| β-mercaptoethanol | 0.5 µL | 1 µL | 2 µL |
| Sample | 50 µL | 100 µL | 200 µL |
| Proteinase K | 20 µL | 40 µL | 80 µL |
| Isopropanol | 85 µL | 170 µL | 340 µL |
| Binding Enhancer | 1.5 µL | 3 µL | 6 µL |
| Magnetic Nanoparticles | 5 µL | 10 µL | 20 µL |

**Caution:** avoid mixing proteinase K with Lysis/Binding solution before sample.

2. Vortex the solution well for 5 seconds, then incubate the mixture at 45°C for 15 minutes with shaking at moderate-high speed.

**Note**: This step can be done in an alternative way if thermal shaker not available – During the incubation, manually flick the tube for more than 10 times every 3 minutes.

3. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on the magnetic rack for 2 minutes, or until the solution clears and the nanoparticles pellet against the magnet.

4. Carefully remove the supernatant with pipette.

### B. Wash nucleic acid with Wash Solution

5. Remove the tube from the magnetic rack, add 400 µL of the prepared Wash Solution. Vortex for 30 seconds to resuspend the nanoparticles.

6. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on the magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

7. Carefully remove the supernatant with pipette.

8. Repeat step 5 – 7 for a second wash.

### C. Wash nucleic acid with 80% ethanol

9. Remove the tube from the magnetic rack, add 400 µL of the prepared 80% ethanol. Vortex for 30 seconds to resuspend the nanoparticle.

10. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on the magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

11. Carefully remove the supernatant with pipette.

12. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on the magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

13. Remove any residual liquid at the bottom of the tube.

### D. DNase treatment and RNA rebinding

14. Remove the tube from the magnetic rack. Add 100 µL of DNase buffer and 1 µL of DNase to the tube, then fully resuspend the nanoparticles by vortexing.

15. Incubate the solution for 10 minutes at 37°C.

16. Remove the tube from the shaker. Add 215 µL of the prepared Re-binding Solution to the tube, then incubate the solution for 8 minutes at room temperature with shaking at moderate-high speed.

### E. Wash RNA with 80% ethanol

17. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

18. Carefully remove the supernatant with pipette.

19. Remove the tube from the magnetic rack, add 400 µL of the prepared 80% ethanol, vortex for 30 seconds to resuspend the nanoparticle.

20. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

21. Carefully remove the supernatant with pipette.

22. Repeat step 19-21 for a second wash.

### F. Elute RNA from magnetic nanoparticles

23. Remove the tube from the magnet, and briefly centrifuge the tube to bring solution to the bottom. Place the tube on magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnet.

24. Remove any residual liquid at the bottom of the tube.

25. Keep the tube on the magnet, air dry the nanoparticles for 3 minutes.

**Note:** If the environment humidity is high, extend the drying time to minimize residual ethanol, which can adversely affect the elution efficiency. Do not over dry. Pellet should not become so dry that it cracks.

26. Remove the 1.5 mL tube from the magnetic rack, then add 50 - 100 µL of RNA Elution Solution to the tube.

27. Vortex the tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 3 minutes to elute the RNA from the nanoparticles.

28. Centrifuge the tube briefly using a tabletop centrifuge to bring solution to the bottom, place the tube on a magnetic rack, wait until the solution clears and the nanoparticles pellet against the magnets.

29. Transfer the supernatant, containing the RNA, to a new DNase/RNase-free and nonstick microcentrifuge tube. Store the RNA sample at -80°C.