Apostle MiniEnrich Short Fragments Enrichment Kit Manual, 20 uL x 50 Preps

Cat#: A190925-50, Version: P.01

Product description

The Apostle MiniEnrich™ Short Fragments Enrichment Kit is designed for enrichment of short DNA fragments from the mixture of DNA with various sizes, which increases the short fragment fraction in the sample and potentially benefits downstream assays. It can be used to enrich short fragments in isolated DNA, library preparation product, PCR amplification product, etc. With proprietary Apostle MiniEnrich™ technology, the kit is featured for customized cut-off size ~200 bp, and the efficient recovery of short fragments of interest as low as 20 bp.

Kit capacity

The kit is capable of enrichment for 20 uL x 50 samples.

Kit contents and storage condition

Contents	Amount	Storage
R Beads Solution*	2 mL	2-8 °C
E Beads Solution*	850 uL	
Elution Solution	3 mL	2-30 °C
Binding Enhancer**	500 uL	-20 °C

* R Beads Solution and E Beads Solution should be brown solution. Vortex to fully resuspend the nanoparticles before use.

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

Required materials not supplied

Adjustable micropipetteors (1 mL, 200 uL, 20 uL) and tips
Magnets specifically designed 2 mL tubes
Nonstick, DNase/RNase-free tubes
Vortex
Shaker
Isopropanol
Ethanol, 200 proof
Ultrapure DNase/RNase free water

Procedure for Short Fragments Enrichment

A. Remove Long Fragments

1. Apostle MiniEnrich™ R Beads Solution should be brown solution. Equilibrate the vial to room temperature and vortex to fully resuspend the nanoparticles before use.

2. Add R Beads Solution to the samples in a 1.5 mL tube. The volume of R Beads Solution for a given reaction can be derived from the following equation:
   Volume of R Beads Solution = Ratio × Sample Volume,
   where the ratio is chosen by the user to select the cut-off size for optimal enrichment effect.

   The cut-off size is affected by the ratio as well as the sample, but as a general rule, higher ratio of R Beads Solution offers smaller cut-off size. An example of the ratio and cut-off size for samples in Tris-EDTA buffer is given as reference:

Cut-off Size	240 bp	200 bp	160 bp	140 bp
Suggested R Beads Solution Ratio	1.0x	1.2x	1.6x	1.8x

   In this protocol, 1600 bp cut-off (1.6x) is used as the demonstration, where the R Beads volume is indicated in the table below:

Reagents	Sample Volume		
	20 uL	40 uL	60 uL
1.6x R Beads Solution*	32 uL	64 uL	96 uL

3. Vortex the solution well for 5 seconds, and incubate the mixture at room temperature for 5 minutes.

4. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on magnet for 3 min, or until the solution clears and the beads are pelleted against the magnet.

5. Carefully transfer the supernatant to a new 1.5 mL tube.

B. Recover Short Fragments

6. Add binding enhancer (**Brown Cap**) and E Beads Solution (**Green Cap**) to the supernatant according to the table below, and mix well by vortexing for 5 s. Briefly centrifuge the tube to bring solution to the bottom.

Sample Volume	20 uL	40 uL	60 uL
Binding Enhancer	8 uL	16 uL	24 uL
E Beads Solution	15 uL	30 uL	45 uL

7. Add 1.22 volume of isopropanol to 1 volume of the mixture in Step 6 . The final concentration of isopropanol is 55%. Thoroughly mix by vortexing briefly. An example of calculated volume of isopropanol based on 1.6x R Beads Solution ratio (160 bp cut-off) is given as reference below:

Volume	Sample Volume		
	20 uL	40 uL	60 uL
Sample	20 uL	40 uL	60 uL
Buffer (from R-beads solution)	32 uL	64 uL	96 uL
Binding Enhancer	8 uL	16 uL	24 uL
E Beads Solution	15 uL	30 uL	45 uL
Total Volume from Step 6	75 uL	150 uL	225 uL
Isopropanol (based on calculated total volume)	92 uL	183 uL	275 uL

8. Shake at moderate-high speed for 10 minutes.
9. Briefly centrifuge the tube to bring solution to the bottom. Place the tube on magnet for 3 min, or until the solution clears and the beads are pelleted against the magnet.
10. Carefully remove the supernatant.

C. Wash with 80% Ethanol

11. Remove the 1.5 mL tube from the magnet, add 1 mL 80% ethanol (made by mixing pure ethanol with ultrapure & DNase/RNase free water, at 4:1 ratio), then vortex for 30 seconds.
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 2 min, or until the solution clears and the nanoparticles are pelleted against the magnets.
13. Remove the supernatant carefully using pipette.
14. Repeat step 11-13 for a second wash.
15. 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.
16. Remove any liquid left in the bottom of 1.5 mL tube.
17. 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.)

D. Elute Short Fragments

18. Remove the 1.5 mL tube from the magnet, add Apostle MiniEnrich™ Elution Solution to the 1.5 mL tube, according to the following table, based on initial sample volume. RNase-free water can also be used as elution solution.

Sample Volume	20 uL	40 uL	60 uL
Suggested Elution Solution Volume	20 uL	40 uL	60 uL

19. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then vortex for another 5 minutes to elute the short fragments from the nanoparticle.
20. 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.
21. Collect the supernatant that contains short fragments in a non-stick, DNase and RNase free microcentrifuge tube. Store the sample at -20 °C for long term strorage.

E. (Optional) Recover Long Fragments

If long fragment is also of intrest, it can be recovered by performing similar washing and elution in section C and D after step 5:

After transfering the supernatant in step 5, take the 1.5 mL tube that contains the R Beads to perform washing in Section C and Elution in section D from step 11 to 21.