

Apostle MiniEnrich™ Size Selection Beads Manual

Cat#: A190606, Version: P.02

Product description

The Apostle MiniEnrich™ Size Selection Beads is designed for size selection of DNA fragments from a mixture of DNA with various sizes, which potentially benefits downstream assays like NGS and other molecular biology workflows. It can be used on samples before or after DNA fragmentation, ligation, or library amplification during NGS library preparation workflow. With proprietary Apostle MiniEnrich™ technology, the kit is featured for customized cut-off size, efficient removal of non-target fragments and recovery of target DNA fragments.

Storage condition

Apostle MiniEnrich™ Size Selection Beads should be brown solution. Upon arrival, store at 2-8 °C. Equilibrate to room temperature and vortex to fully resuspend the nanoparticles before use.

Required materials not supplied

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

Procedure for Size Selection

This protocol provides a guideline to perform size selection, where user can choose desired approach and cut-off size based on specific applications.

- Section A: Left side selection, for recovery of fragments longer than a cut-off size
- Section B: Right side selection, for recovery of fragments shorter than a cut-off size
- Section C: Double side selection, for recovery of fragments between a lower and higher cut-off size.

The cut-off size is affected by the volume ratio of beads to sample as well as the input sample, but as a general rule, higher volume ratio increases the binding capability of smaller fragments of the beads.

A. Left side size selection

1. Before use, equilibrate Apostle MiniEnrich™ Size Selection Beads to room temperature and vortex to fully resuspend the beads.
2. Add Size Selection Beads to the samples based on the desired cut-off size and sample volume. The volume of beads for a given reaction can be derived from the following equation:

$$\text{Volume of beads} = \text{Ratio} \times \text{Initial Sample Volume.}$$

An example of the ratio and cut-off size for samples in Tris-EDTA buffer is given as reference:

| Cut-off Size | 250 bp | 300 bp | 400 bp | 600 bp |
|-----------------------|--------|--------|--------|--------|
| Suggested Beads Ratio | 0.9x | 0.8x | 0.7x | 0.6x |

3. Vortex the mixture 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 remove the supernatant.
6. Remove the 1.5 mL tube from the magnet, add 500 uL 80% ethanol (made by mixing pure ethanol with ultrapure & DNase/RNase free water, at 4:1 ratio), then vortex for 30 seconds.
7. 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.
8. Remove the supernatant carefully using pipette.
9. Repeat step 6-8 for a second wash.
10. 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.)

11. Remove the 1.5 mL tube from the magnet, add DNase/RNase free water to elute DNA. We suggest using ≥ 20 μ L or same volume as the input sample of DNase/RNase free water for efficient elution.
12. Vortex the 1.5 mL tube to resuspend the magnetic nanoparticles in the solution, then incubate for another 5 minutes to elute the DNA fragments from the nanoparticle.
13. 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.
14. Collect the supernatant that contains DNA fragments of desired size in a non-stick, DNase and RNase free microcentrifuge tube. For long term storage, store the sample at -20 $^{\circ}$ C.

B. Right side size selection

1. Before use, equilibrate Apostle MiniEnrich™ Size Selection Beads to room temperature and vortex to fully resuspend the beads.
2. Add Size Selection Beads to the samples based on the desired cut-off size and sample volume. The volume of beads for a given reaction can be derived from the following equation:

$$\text{Volume of beads} = \text{Ratio} \times \text{Initial Sample Volume.}$$

An example of the ratio and cut-off size for samples in Tris-EDTA buffer is given as reference:

| Cut-off Size | 250 bp | 300 bp | 400 bp | 600 bp |
|-----------------------|--------|--------|--------|--------|
| Suggested Beads Ratio | 0.9x | 0.8x | 0.7x | 0.6x |

3. Vortex the mixture 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.
6. Add Size Selection Beads to the supernatant based on the initial ratio in step 2 of Section B. The volume of beads for a given reaction can be derived from the following equation:

$$\text{Volume of beads} = (1.8 - \text{Initial Ratio}) \times \text{Initial Sample Volume}$$

7. Vortex the mixture for 5 seconds, and incubate the mixture at room temperature for 5 minutes.
8. 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.
9. Carefully remove the supernatant.
10. Follow step 6-14 in Section A to wash and elute the fragments shorter than the cut-off size.

C. Double side size selection

1. Before use, equilibrate Apostle MiniEnrich™ Size Selection Beads to room temperature and vortex to fully resuspend the beads.
2. Add Size Selection Beads to the samples based on the desired higher cut-off size and sample volume. The volume of beads for a given reaction can be derived from the following equation:

$$\text{Volume of beads} = \text{Initial Ratio} \times \text{Initial Sample Volume}$$

An example of the ratio and cut-off size for samples in Tris-EDTA buffer is given as reference:

| Higher Cut-off Size | 250 bp | 300 bp | 400 bp | 600 bp |
|-------------------------|--------|--------|--------|--------|
| Suggested Initial Ratio | 0.9x | 0.8x | 0.7x | 0.6x |

3. Vortex the mixture 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.
6. Add Size Selection Beads to the supernatant based on the initial ratio in step 2 of Section C. The volume of beads for a given reaction can be derived from the following equation:

$$\text{Volume of beads} = (\text{Second} - \text{Initial Ratio}) \times \text{Initial Sample Volume,}$$

where the second ratio is chosen by the user to determine the lower cut-off size. An example of the ratio and lower cut-off size for samples in Tris-EDTA buffer is given as reference:

| Lower Cut-off Size | 250 bp | 300 bp | 400 bp | 600 bp |
|------------------------|--------|--------|--------|--------|
| Suggested Second Ratio | 0.9x | 0.8x | 0.7x | 0.6x |

7. Vortex the mixture for 5 seconds, and incubate the mixture at room temperature for 5 minutes.
8. 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.
9. Carefully remove the supernatant.
10. Follow step 6-14 in Section A to wash and elute the fragments between the lower and higher cut-off sizes.