AFTMag NGS DNA Clean Beads

RK20257

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1. Introduction

ABclonal AFTMag NGS DNA Clean Beads (RK20257) utilize SPRI (Solid Phase Reverse Immobilization) paramagnetic bead technology to enable DNA purification and size selection during NGS library construction. AFTMag NGS DNA Clean Beads are compatible with most NGS library construction kits (DNA, RNA). The kit allows users to seamlessly replace competitor beads (i.e. AMPure XP Beads) in current bead clean-up and size selection protocols without loss of efficiency, while effectively reducing library construction costs.

2. Precautions

The shelf life of kit components is two years when all reagents are stored at 4℃ and protected against exposure to light.

3. Additonal Materials Required

- 80% ethanol
- Nuclease-free ddH_2O
- PCR tubes
- Magnetic frame
- Filtered pipette tips
- Micro-centrifuge
- Vortex mixer
- Single-channel pipette and multi-channel pipette
- Agilent Bioanalyzer or comparable method to assess the DNA library quality

4. Protocol

4.1 DNA Purification

4.1.1 Allow the magnetic bead solution to equilibrate to room temperature for 30 minutes.

4.1.2 Vortex to fully resuspend the magnetic bead solution. According to Table 1, transfer the indicated amount of bead mixture to the DNA sample in a microcentrifuge tube and gently mix with a pipette.

4.1.3 Incubate at room temperature for 5 min to bind DNA to magnetic beads.

4.1.4 Place the sample on a magnetic frame and carefully remove the supernatant after the solution is clarified (about 5 min).

4.1.5 With the sample still on the magnetic frame, rinse the magnetic beads with 200ul freshly-prepared 80% ethanol. Incubate at room temperature for 30 sec to clarify and carefully remove the supernatant.

4.1.6 Repeat step 4.1.5 once for a total of two ethanol rinses.

4.1.7 With the sample on the magnetic frame, open the microtube cover and airdry the magnetic beads at room temperature for about 2 - 3 min.

4.1.8 Remove the sample from the magnetic frame, add an appropriate amount of Nuclease-free ddH_2O, gently mix with a pipette, and let stand at room temperature for 2 min.

4.1.9 Return the reaction tube to the magnetic frame for 1 min to separate beads from the solution. Transfer supernatant to a new nuclease free centrifuge tube after sample is transparent.

Table 1. Reference conditions for DNA purification

Fragment size range after purification (bp)	Reference purified magnetic bead dosage (magnetic bead volume dosage: sample volume)
≥1 kb	0.5X
≥400 bp	0.8X
≥300 bp	1.0X
≥200 bp	1.5X
≥100 bp	2.2X

4.2 DNA classification selection

4.2.1 Allow the magnetic bead solution to equilibrate to room temperature for 30 minutes.

4.2.2 Vortex to fully resuspend the magnetic beads. According to the sorting conditions of the library preparation kit (see Table 2) pipette appropriate volume of magnetic bead liquid (Table 2, 1st volume ratio) and add it to the purified DNA sample and gently mix with the pipette.

4.2.3 Incubate at room temperature for 5 min to bind DNA to magnetic beads.

4.2.4 Place the sample on a magnetic frame until the solution is clarified (about 5 min). Carefully remove the supernatant and transfer to a new nuclease free centrifuge tube for secondary sorting. (The supernatant should be retained)

4.2.5 With a pipette, add the indicated amount of magnetic bead liquid (Table 2, 2nd volume radio) and mix thoroughly by pipetting.

4.2.6 Incubate at room temperature for 5 min to bind DNA to magnetic beads.

4.2.7 Place the sample on a magnetic frame and carefully remove the supernatant after the solution is clarified (about 5 min).

4.2.8 With the sample on the magnetic frame, add 200 µl of freshly prepared 80% ethanol to rinse the beads, incubate at room temperature for 30 sec, and carefully remove the supernatant.

4.2.9 Repeat step 4.2.8 once and rinse twice in total.

4.2.10 With the sample on the magnetic frame, open the microtube cover and airdry the magnetic beads at room temperature for about 2 - 3 min.

4.2.11 Remove the sample from the magnetic frame, add an appropriate amount of Nuclease-free ddH_2O, and mix with a pipettor or vortex mixer. Let stand at room temperature for 2 min, and then place the reaction tube on the magnetic frame for 1 min.

4.2.12 Transfer the supernatant to a new nuclease free centrifuge tube after sample is transparent.

Table 2. Recommended condition for DNA fragment sorting

Average length range of sorted fragments (bp)	300bp	350bp	400bp	500bp	600bp	650bp
1st volume ratio (Beads: DNA)	0.80x	0.70x	0.60x	0.55x	0.50x	0.45x
2nd volume ratio (Beads: DNA)	0.20x	0.20x	0.20x	0.15x	0.15x	0.15x

5. Notes

5.1 To ensure high efficiency of DNA recovery, please remove the AFTMag NGS DNA Clean Beads from 2 ~ 8℃ 30 min in advance and balance to room temperature. To ensure uniform mixing, please vortex reagent before use.

5.2 When washing the sample with 80% ethanol, keep the sample tube on the magnetic frame and do not disturb the magnetic beads. Drying time may vary to ensure all traces of ethanol are removed. Avoid over-drying the bead ring (bead ring appears cracked if over dried) as it will significantly decrease elution efficiency.

5.3 When using Agilent 2100 Bioanalyzer to analyze samples, there may be tailing at higher molecular weight ranges, which is typically caused by the residue of trace magnetic beads. A magnetic frame with strong magnets and avoiding disturbing the beads at the final step can decrease magnetic bead carryover.

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