sparQ PureMag Beads

Cat. No.	95196-005	Size:	5 mL	**Store at 2°C to 8°C**
	95196-060		60 mL	
	95196-450		450 mL	

Description

sparQ PureMag Beads uses reversible nucleic acid-binding properties of magnetic beads for fast and efficient nucleic acid purification. It is an ideal choice for reaction cleanup during NGS library preparation to remove primers, primer-dimers, unincorporated nucleotides, salts, adapters and adapter-dimers. This product can also be used for DNA size selection and provides consistent size range by simply manipulating the beads to sample ratio. Compatible with both manual and automated purification of nucleic acids, sparQ PureMag Beads can substitute other similar products with little or no change to the existing protocol or the instrument setting.

Instrument Compatibility

This product is compatible with automation platforms.

Storage and Stability

Store the product in a constant temperature refrigerator at 2°C to 8°C upon receipt. Please do not freeze.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Additional reagents and materials that are not supplied

- Magnetic separation device
- Vortexer
- Pipettors
- Freshly-prepared 80% ethanol
- Elution Buffer (10 mM Tris pH 8.0), TE Buffer, or nuclease-free water

General Guidelines

Use good laboratory practices to minimize cross-contamination of nucleic acid products.
Always use PCR tubes, microfuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.

Before You Begin

Equilibrate the sparQ PureMag Beads to room temperature (RT) prior to use.
Fully resuspend the beads in homogenous form before use.
Always prepare fresh 80% ethanol prior to use.

Protocol

1. Reaction Cleanup in NGS Workflows

There are several sample purification steps in NGS library preparation workflow, including post adapter ligation and library amplification. sparQ PureMag Beads can be used in all those steps. We have demonstrated that the sparQ PureMag Beads can be directly used to replace AMPure® XP beads when using any of Quantabio sparQ DNA library preparation kits, and no protocol change, or additional optimization is required. Below are examples of how to use sparQ PureMag Beads for each purification step involved during library preparation using sparQ kits.

A. Removal of Divalent Cations and EDTA from Input Nucleic Acid

Input DNA for the fragmentation reaction should be in water, 10 mM Tris, buffer EB, or LoTE (0.1X TE). If the DNA was dissolved in 1X TE or the concentration of cations and chelators is not known, we strongly recommend purifying the DNA per instructions below.

1. If DNA is in a volume of less than 50 µl, adjust the volume to 50 µl with nuclease-free water.
2. Equilibrate the sparQ PureMag Beads to RT for at least 20 min.
3. Add 90 µl of thoroughly vortexed and equilibrated beads to the reaction for a ratio of 1.8X and mix well by pipetting. If DNA is in a volume greater than 50 µl, scale the volume of the beads appropriately such that the ratio of beads to DNA is 1.8X.
4. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand for 2-4 min and carefully discard the supernatant without disturbing the beads.
5. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
6. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads are dry.
7. Thoroughly resuspend the dried beads in 45 µl of 10 mM Tris-HCl, pH 8.0 and incubate for 2 min at RT. Pellet the beads on the magnetic stand and carefully transfer 42.5 µl of supernatant into a new tube.
8. Determine the concentration of the purified DNA using Qubit, PicoGreen or other methods.

B. Reaction Cleanup after Adapter Ligation

The optimal beads to DNA sample ratio for DNA fragments in the >150 bp size range is 0.8X. Conditions may differ if the desired DNA size range is different.

1. If DNA is in a volume of less than 50 µl, adjust the volume to 50 µl with nuclease-free water.
2. Equilibrate the sparQ PureMag Beads to RT.
3. Vortex the beads thoroughly and add 80 µl (0.8X) to reaction tube. Mix well by pipetting.
4. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag®) and carefully discard the supernatant.
5. Wash the beads with 200 µl of the freshly-prepared 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
6. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.
7. Resuspend the dried beads in 25.5 µl of 10 mM Tris-HCl, pH 8.0 and incubate for 2 min at RT. Pellet the beads on the magnetic stand. Carefully transfer 23.5 µl of supernatant into a new tube. The sample can be stored at -20°C if not proceeding immediately to library amplification or other downstream processes.

C. Post PCR-Amplification Cleanup

1. If DNA is in a volume of less than 50 µl, adjust the volume of 50 µl with nuclease-free water.
2. Equilibrate the sparQ PureMag beads to RT.
3. Thoroughly vortex the beads slurry and add 50 µl (1X) to the PCR reaction. Mix well by pipetting.
4. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag) and carefully discard the supernatant.
5. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
6. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.
7. Resuspend the dried beads in 32.5 µl of 10 mM Tris-HCl, pH 8.0 and incubate for 2 min at RT. Pellet the beads on the magnetic stand. Carefully transfer 30 µl of supernatant into a new tube. The sample can be stored at -20°C if not proceeding immediately to library quantification or other downstream processes.

2. Size Selection in NGS library Preparation

Depending on the specific application, size selection may be required to ensure the DNA insert size falls into a certain range. Size selection can be implemented in different steps, such as after DNA fragmentation, post-ligation cleanup, or after library amplification. Because the reaction buffer, especially the ligation buffer, can greatly influence the size selection range, it is recommended to perform size selection after an initial purification using the sparQ PureMag Beads (see section 1A-1C for detailed instruction). If size selection without initial purification is preferred, the exact amount of beads used needs to be determined empirically.

The following protocol and guidelines are based on double-sided size selection of fragmented DNA without Y-shaped adapters attached. Additional optimization is required if using adapter ligated DNA or post-PCR DNA. The size range reflects the final fragment size, so if calculating the insert size, please take into account that the adapter size and configuration both have a significant impact on the size of the fragment appearing on the Bioanalzyer or other size differentiation and detection instruments. Example below shows the double-sided size selection of DNA sample in a 50 µl volume, and the targeted size selection range of 200-400 bp (centered around 300 bp: Figure 1)

1. If DNA is in a volume of less than 50 µl, adjust the volume to 50 µl with nuclease-free water.
2. Equilibrate the sparQ PureMag Beads to RT for at least 20 min.
3. Thoroughly resuspend the beads.
4. Vortex the beads and add 35 µl (0.7X) to reaction tube. Mix well by pipetting.
5. Incubate the mixture for 5 min at RT. Pellet the beads on the magnetic stand until the liquid is clear. Do not discard supernatant.
6. Transfer all supernatant to a new tube. Be careful not to carry any beads into the new tube.
7. Add 10 µl of new beads into the tube contains the supernatant and mix thoroughly by vortexing or pipetting up and down several time with Pipette set at 100 µl. To ensure the accurate amount of beads is added, take extra caution during pipetting and mixing as the solution can be viscous to handle.
8. Pellet the beads on the magnetic stand. Carefully discard the supernatant after the liquid is clear.
9. Keep the tube on the magnetic stand and wash the beads with 200 µl of the freshly-prepared 80% ethanol.
10. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
11. Air-dry the beads on the magnetic stand for 3 - 5 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.
12. Resuspend the dried beads in desired volume of 10 mM Tris-HCl, pH 8.0 or water and incubate for 2 min at RT. Pellet the beads on the magnetic stand. Carefully transfer the eluent into a new tube.

General guideline for double-sided size selection of various size-range of DNA fragments

	Final DNA fragment size range		
	200-400 bp	250-500 bp	300-700 bp
1st bind beads amount	35 µl	30 µl	25 µl
2nd bind additional beads amount	10 µl	10 µl	10 µl

The volume of beads added was calculated based on starting DNA in 50 µl of Tris buffer or water.

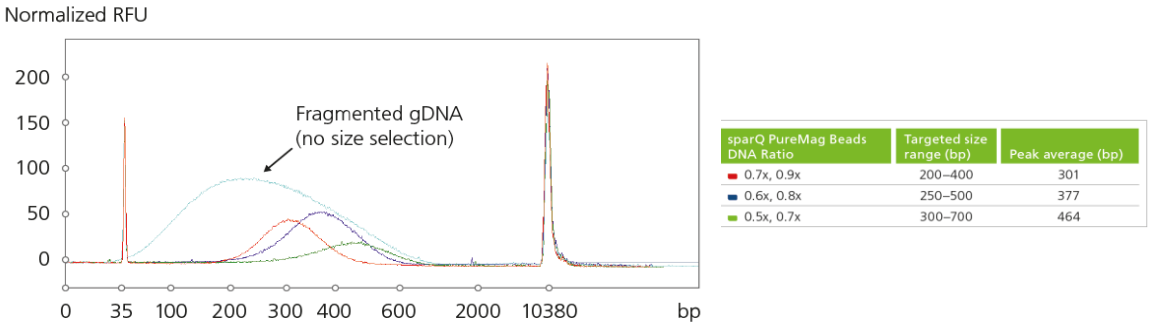

Figure 1: Example of genomic DNA fragment before (bright blue) and after double-sided size selection in the range of 200- 700 bp.

Quality Control

All kit components are subjected to quality control, including DNA recovery assay, DNA size selection assay (specific size range), RNA Recovery Assay, and PCR Inhibition Assay.

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Related NGS Products Sold Separately

sparQ DNA Frag and Library Prep Kit

Cat. No.	95194-024	Size:	24 reactions
	95194-096		96 reactions

The sparQ DNA Frag & Library Prep Kit is optimized for enzymatic fragmentation of DNA and streamlined construction of high quality libraries for sequencing on NGS platforms. The simple, convenient 2-step workflow can be completed in 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 1 ng to 1000 ng.

sparQ DNA Library Prep Kit

Cat. No.	95191-024	Size:	24 reactions
	95191-096		96 reactions

The sparQ DNA Library Prep Kit is optimized for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on NGS platforms. The simplified protocol speeds up library prep to 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 250 pg to 1 µg.

sparQ HiFi PCR Master Mix

Cat. No.	95192-050	Size:	50 reactions
	95192-250		250 reactions

The sparQ HiFi PCR Master Mix is a high efficiency, high-fidelity, and low bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 Illumina® flow cell sequences.

sparQ Universal Library Quant Kit

Cat. No.	95210-100	Size:	100 reactions
	95210-500		500 reactions

The sparQ Universal Library Quant Kit is optimized for rapid, sensitive, and accurate quantification of Illumina® NGS libraries of various sizes and GC-contents. The kit uses fast cycling protocol, allowing results to be achieved in 40 minutes versus 1 hour and 20 minutes with other NGS library quantification kits.