sparQ PureMag Beads Room Temperature Stability

Keywords: sparQ PureMag Beads, stability, DNA, cleanup, size selection, room temperature

ABSTRACT

The need for cold storage of molecular biology reagents to ensure stability and performance is common. For example, sparQ PureMag Beads, which can be used for DNA and RNA cleanup in a wide range of applications, are recommended to be stored at 4°C. In this application note we demonstrate stability of sparQ PureMag Beads at room temperature for up to six months. Performance of beads stored at room temperature or at 4°C was comparable, demonstrating that sparQ PureMag Beads are tolerant to storage at ambient temperatures.

INTRODUCTION

sparQ PureMag Beads provide an efficient, reliable method for DNA or RNA cleanup for a variety of applications including: PCR cleanup, next generation sequencing library preparation and cDNA synthesis. The beads are manufactured to high standards to ensure batch to batch consistency, reproducibility and exceptional stability. The beads are shipped at ambient temperature but it is recommended that the beads are stored at 4°C to maintain these properties. However, some applications require that beads are stored at room temperature for extended periods of time, for example during transport or when used with workflow automation instruments. In this application note, we conducted real-time stability testing of sparQ PureMag Beads at room temperature for up to six months.

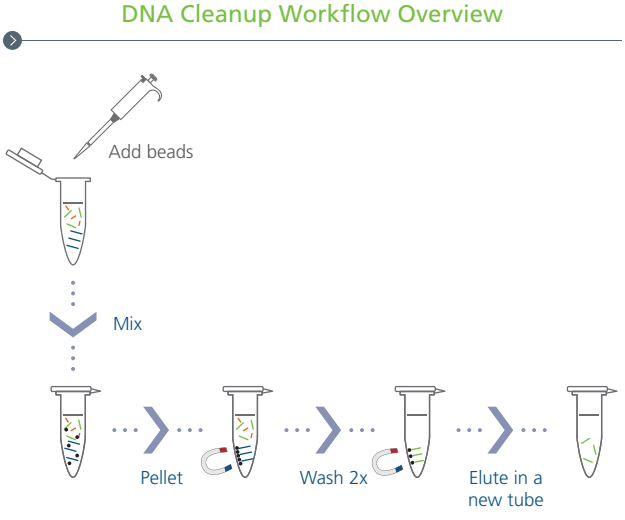

Figure 1 **DNA cleanup workflow overview.** (1) Beads are added to the DNA sample and mixed. (2) The beads are pelleted and the supernatant discarded. (3) The pellet is held on the magnet and washed twice. (4) The DNA is eluted in 10 mM Tris·HCl pH 8.0.

We found that room temperature storage of sparQ PureMag Beads had no impact on the performance of the beads, efficiency of DNA recovery or size selection.

METHODS

Bead storage

Two aliquots of sparQ PureMag Beads were prepared from a single lot and stored at room temperature or 4°C for six months before use. For each experiment, beads stored at 4°C were equilibrated to room temperature for 20 min before use. Both bead aliquots were vortexed thoroughly to resuspend beads before use.

DNA cleanup using sparQ PureMag Beads

50 bp DNA Ladder (NEB #N3236L) was used as a model sample containing fragments of various lengths. The DNA ladder was diluted to 20 ng/µl and 50 µl (1000 ng) of this diluted stock used as the input DNA for each experiment.

DNA cleanup experiments were carried out in triplicate as follows:

1) sparQ PureMag Beads were added to the DNA sample at the specified ratio (0.6X or 1.8X).
2) The DNA/sparQ PureMag Bead mixture was incubated at room temperature for 5 min.
3) The beads were pelleted on a magnet and the supernatant removed and discarded.
4) The bead pellet was then washed twice in 80% EtOH then air dried.
5) DNA was eluted in 35 µl 10 mm Tris·HCl pH 8.0 and collected in a fresh tube.

Analysis

Eluted DNA was quantified using a Nanodrop™ 8000 spectrophotometer (Thermo Scientific). To determine size selection, fragment distribution was analyzed on a D1000 DNA Screen Tape on an TapeStation® 4200 instrument (Agilent Technologies).

RESULTS

High recovery of DNA with sparQ PureMag Beads stored at room temperature

sparQ PureMag Beads were stored at 4°C or at room temperature for six months, then used for DNA purification. Figure 2 shows average DNA recovery (%) from purification using a 1.8X bead to DNA ratio. DNA recovery was high, regardless of the storage conditions of the beads. Minor loss of DNA during cleanup was expected as the ladder includes 50 bp fragments and the bead cleanup process selected fragments >100 bp. The consistency of DNA recovery was also maintained as each replicate of sparQ PureMag Beads purification produced equivalent DNA recovery from beads stored in either condition. After six months, recovery of DNA using beads stored at 4°C was 84.4%, σ = 2.8 and at room temperature was 84.5%, σ = 1.8.

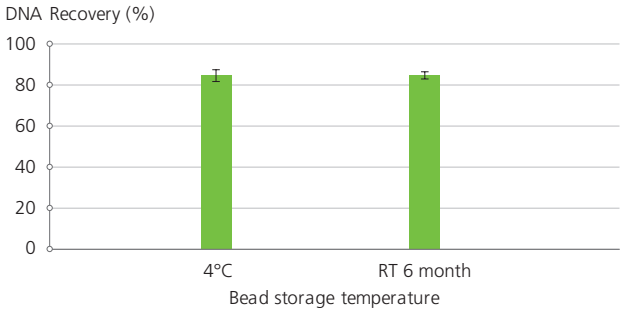

Figure 2 **DNA recovery from cleanup with sparQ PureMag Beads.** Beads were stored at 4°C or room temperature (RT) for six months then used to purify 1000 ng DNA. Eluted DNA concentration was measured and percentage recovery calculated. Bars show mean ± s.d.

Efficient size selection of DNA with sparQ PureMag Beads stored at room temperature

sparQ PureMag Beads provide easy and efficient fragment size selection, for example in NGS workflows, by simply manipulating the beads to sample ratio. To test the effectiveness of sparQ PureMag Beads for size selection after room temperature storage, DNA was purified using a ratio of 0.6X sparQ PureMag Beads. Figure 3 shows size selection using beads stored at 4°C or at room temperature for six months. Size selection was similar using beads stored in either condition, with preferential retention of longer fragments >200 bp and removal of shorter fragments. Total DNA recovery was also comparable, at 60.3%, σ = 1.2, with beads stored at 4°C and 61.4%, σ = 1.0, with beads stored at room temperature for six months.

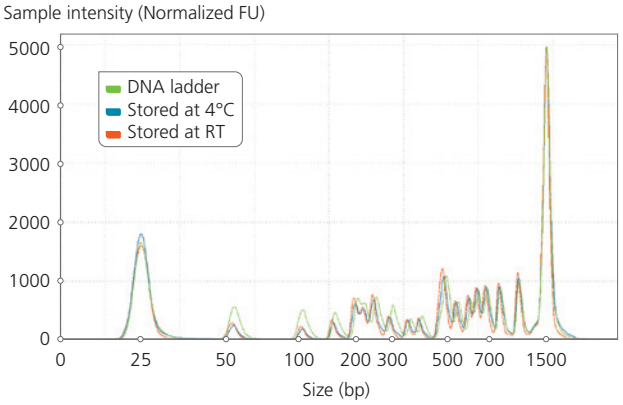

Figure 3 **Size selection of DNA with sparQ PureMag Beads.** Beads were stored at 4°C or room temperature (RT) for six months then used for size selection of 50 bp DNA ladder. Figure shows overlay of representative digital electropherogram images of 50 bp DNA ladder (green), or DNA eluted from size selection using beads stored at 4°C (blue) or room temperature (orange).

For genomics applications, the sterility of reagents is vital to prevent contamination of samples. Analysis of the solution from bead aliquots stored at 4°C or room temperature for 6 months confirmed that there was no microbial growth in either solution. Therefore, storage of beads at room temperature does not infer a contamination risk from the beads.

CONCLUSIONS

In this application note, we have demonstrated equivalent DNA recovery and size selection by sparQ PureMag Beads stored at 4°C or at room temperature. Tolerance to provisional storage at ambient temperatures improves procedural flexibility for situations that require extended exposure to higher temperatures and confidence that inadvertent storage errors do not affect performance. For maximum long term stability, storage at 4°C is recommended, however, the data presented here demonstrate that room temperature storage for six months does not negatively impact cleanup of DNA using sparQ PureMag Beads.

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