sbeadex SAB
(sequencing application beads)
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1. Introduction

The sbeadex™ sequencing application beads (SAB) kit combines the requirement for purified high quality DNA fragments with the superior performance of sbeadex beads – LGC, Biosearch Technologies’ proprietary double-coated magnetic beads. Depending on the sbeadex SAB mix concentration, the chemistry purifies distinct DNA fragments of a defined range, thus facilitating target enrichment based on fragment size. It also efficiently removes contaminants including salts, free dNTPs and primers/primer dimers. sbeadex SAB kits can be used for both manual and automated (e.g. ThermoFisher™ KingFisher™) purifications. This kit is intended for research use applications only. It is not intended for use in diagnostic procedures.

The data presented here illustrates protocols from pre-sheared DNA. The guidance below can be used to optimise the desired size selection range based on the sheared DNA used for selection.

2. Kit contents and storage conditions

Please see Table 1 for the scales of the sbeadex SAB kits which can be ordered:

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Volume of sbeadex SAB chemistry</th>
<th>Number of preparations per kit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP45001</td>
<td>5 mL</td>
<td>133</td>
</tr>
<tr>
<td>NAP45002</td>
<td>20 mL</td>
<td>533</td>
</tr>
<tr>
<td>NAP45003</td>
<td>100 mL</td>
<td>2,666</td>
</tr>
<tr>
<td>NAP45004</td>
<td>450 mL</td>
<td>12,000</td>
</tr>
</tbody>
</table>

Table 1: Ordering information. *The number of preparations is based on Biosearch Technologies' standard protocol that assumes 50 μL starting volume of DNA, and a dual sided size selection protocol (700 bp and 200 bp) being performed. For Research Use Only. Not for use in diagnostic procedures.

All kit components should be used by the expiry date stated on the kit box, and stored under the recommended storage conditions. Please see Table 2 for details.

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>sbeadex particles suspension sequencing application beads (SAB)</td>
<td>4 °C</td>
</tr>
<tr>
<td>Elution Buffer PN</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

Table 2: sbeadex SAB kit components and storage conditions.

3. Experimental procedure

3.1 General information before starting

When performing the sbeadex SAB kit protocol, a magnet or centrifuge is required to pellet the magnetic particles.

If performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds (single tubes: full speed; plates: 2,000 x g) to enable the magnetic particles to form a pellet. All processes are to be carried out at room temperature (15 °C–25 °C).
For information on automation of the sbeadex SAB kit protocol, see Section 4 of this manual.

The following technical definitions and considerations (Table 3) should be applied to the experimental process, each time they are stated:

<table>
<thead>
<tr>
<th>Technical description</th>
<th>Consideration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bring beads to room temperature</td>
<td>In order to get optimal performance and proper size selection with sbeadex SAB, the beads must be at room temperature before being combined with the DNA samples.</td>
</tr>
<tr>
<td>Bring magnet into contact with tubes</td>
<td>This will allow the sbeadex SAB to form a pellet on the side of the tube, to allow for easy removal of the supernatant. The times stated for sbeadex SAB pelleting are minimum recommended incubation times. The strength of the magnet will influence the speed of sbeadex SAB pelleting, and so an increased incubation time should be used, if required, to ensure all beads are pelleted.</td>
</tr>
<tr>
<td>Periodic mixing (optional)</td>
<td>The sample can be periodically mixed by pipetting/vortexing/shaking to ensure the sbeadex particles remain in suspension. This mixing may increase the efficiency of the binding/washing.</td>
</tr>
<tr>
<td>Mix thoroughly</td>
<td>The sample should be mixed by pipetting, to ensure the sbeadex SAB are completely resuspended. The mixing can be assisted by pipetting or vortexing in 5-10 second bursts.</td>
</tr>
<tr>
<td>Removal of supernatant</td>
<td>When removing supernatant, it is important to remove as much liquid as possible without dislodging the particle pellet. When placing the pipette tip inside the tube, ensure that the tip is aimed towards the front wall of the sample tube to avoid disruption of the particle pellet. It is recommended to aspirate once, let any liquid run down the walls of the tube, and then aspirate a second time to remove any remnants of liquid.</td>
</tr>
<tr>
<td>Store beads protected from light</td>
<td>We recommend protecting the beads from light when bringing to room temperature. As such, we recommend mixing the original bottle thoroughly, removing an aliquot for the experiments you are performing, and protecting that aliquot from light (e.g. wrap in foil). Then return the original bottle of beads to its 4 °C storage protected from light.</td>
</tr>
</tbody>
</table>

Table 3: Technical descriptions in the protocols outlined in this manual and the considerations which should be adhered to when following the methods.

3.2 Required materials (not included)

a) 100% ethanol; molecular biology grade
b) Ultrapure nuclease-free water; molecular biology grade
c) Magnetic rack or centrifuge
d) 96- or 384-well plates, or reaction tubes

3.3 Initial preparations

a) Prepare DNA: The recommended starting DNA volume is at least 50 μL. Should the starting DNA contain contaminants which may interfere with downstream processes, the starting DNA volume can be increased, to dilute out these contaminants. All DNA dilutions should be performed with nuclease-free, molecular biology grade water.

b) Prepare required 75% ethanol (v/v in water): Each reaction requires 400 μL 75% ethanol.

3.4 Sample clean-up

This protocol is suitable for general reaction clean-up. DNA fragments above ~100 bp will be purified, whilst smaller DNA fragments such as primers will be removed. Reaction components such as buffers, enzymes, dNTPs etc. will also be removed. If purification of DNA below/above a certain size is required, or within a size range, please see Section 3.5, Section 3.6 and Section 3.7, respectively.
1. Bring beads to room temperature while protecting from light.
2. Thoroughly mix sbeadex SAB to ensure that the beads are fully resuspended.
3. Based on the total volume of the starting DNA, add 1.5x volume of sbeadex SAB to the DNA solution. Ensure starting DNA volume is ≥50 μL.
4. Mix thoroughly and incubate for 5 minutes with periodic shaking.
5. Bring magnet into contact with the tube(s) for 1 minute.
6. Remove the supernatant and discard.
7. Separate the magnet from the sample tubes.
8. Add 200 μL 75% ethanol (v/v in water). Mix thoroughly.
9. Incubate for 2 minutes with periodic shaking (shaking is optional).
10. Bring magnet into contact with the tube(s) for 1 minute.
11. Remove the supernatant and discard.
12. Repeat steps 7–11.
13. Dry bead pellet in open tube for 5 minutes.
15. Incubate for 5 minutes with periodic shaking.
16. Bring magnet into contact with the tube(s) for 1 minute.
17. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.

Fragments of the desired size are bound to the sbeadex SAB.

3.5 Left side size selection—remove DNA below a certain size
This protocol is suitable for the purification of DNA fragments of one selected size and greater; all fragments below the cut-off size will be excluded and all fragments above the cut-off size will be selected. If removal of DNA above a certain size is required, please see Section 3.5. If a specific range of fragment sizes are required (that is excluding both small and large fragments), please follow the protocol detailed in Section 3.7.

For guidance on volumes of sbeadex SAB for left side size selection, please refer to Figure 3 and Table 4.
1. Bring beads to room temperature while protecting from light.
2. Thoroughly mix sbeadex SAB to ensure that the beads are fully resuspended.
3. Based on the desired fragment sizes (see Figure 3 and Table 4), add the corresponding volume of sbeadex SAB to the DNA solution. Ensure total volume of DNA is ≥50 μL.
4. Mix thoroughly and incubate for 5 minutes with periodic shaking.
5. Bring magnet into contact with the tube(s) for 1 minute.
   
   **Fragments of the desired size are bound to the sbeadex SAB.**
6. Remove the supernatant and discard.
7. Separate the magnet from the sample tubes.
8. Add 200 μL 75% ethanol (v/v in water). Mix thoroughly.
9. Incubate for 2 minutes with periodic shaking (shaking is optional).
10. Bring magnet into contact with the tube(s) for 1 minute.
11. Remove the supernatant and discard.
12. Repeat steps 7-11.
13. Dry bead pellet in open tube for 5 minutes.
15. Incubate for 5 minutes with periodic shaking.
16. Bring magnet into contact with the tube(s) for 1 minute.
17. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.

3.6 Right side size selection - remove DNA above a certain size

This protocol is suitable for the purification of DNA fragments of one selected size and smaller; all fragments above the cut-off size will be excluded and all fragments below the cut-off size will be selected. This is a two-stage process. The first stage removes all fragments which are too large, and the second stage captures all DNA below the desired cut-off by utilising a high sbeadex particle ratio (1.5x).
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If removal of DNA below a certain size is required, please see Section 3.5. If a specific range of fragment sizes are required (that is excluding both small and large fragments), please follow the protocol detailed in Section 3.7.

The volume of SAB required for the second binding (that is to capture all small fragments) is calculated using the formula below:

$$[\text{Volume of SAB required for second binding}] = [1.5x \text{ starting DNA volume}] - [\text{Volume of SAB used in first binding}]$$

![Figure 4: Calculation for the volume of SAB required to perform the lower cut-off in right side size selection](image)

For guidance on volumes of sbeadex SAB for right side size selection, please refer to Figure 5 and Table 5.

![Figure 5: Agilent High Sensitivity DNA electropherogram of right side size selections using a range of sbeadex SAB ratios (volume sbeadex/volume DNA) for first stage. Second stage sbeadex particle volume is 1.5x final for all selections. Sheared human genomic DNA input (1 μg in 100 μL 10 mM Tris pH 8.0) is shown in black.](image)

<table>
<thead>
<tr>
<th>sbeadex SAB ratios (volume sbeadex/volume DNA)</th>
<th>Right side DNA fragment size selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A – 0.95x - 1.5x</td>
<td>300 bp</td>
</tr>
<tr>
<td>B – 0.90x - 1.5x</td>
<td>325 bp</td>
</tr>
<tr>
<td>C – 0.85x - 1.5x</td>
<td>350 bp</td>
</tr>
<tr>
<td>D – 0.80x - 1.5x</td>
<td>400 bp</td>
</tr>
<tr>
<td>E – 0.75x - 1.5x</td>
<td>475 bp</td>
</tr>
<tr>
<td>F – 0.70x - 1.5x</td>
<td>500 bp</td>
</tr>
<tr>
<td>G – 0.65x - 1.5x</td>
<td>700 bp</td>
</tr>
<tr>
<td>H – 0.60x - 1.5x</td>
<td>900 bp</td>
</tr>
<tr>
<td>I – 0.55x - 1.5x</td>
<td>1500 bp</td>
</tr>
<tr>
<td>J – 0.50x - 1.5x</td>
<td>1750 bp</td>
</tr>
</tbody>
</table>

Table 5: Right side size selection using a range of sbeadex SAB ratios (volume sbeadex/volume DNA), based on sheared human genomic DNA input (1 μg in 100 μL 10 mM Tris pH 8.0) shown in Figure 5.

![Figure 6: Schematic of right side size selection protocol.](image)
1. Bring beads to room temperature while protecting from light.
2. Thoroughly mix sbeadex SAB to ensure that beads are fully resuspended.
3. Based on the desired fragment size for the upper cut-off (Figure 5 and Table 5), add the corresponding volume of sbeadex SAB to the DNA solution. Ensure the starting volume of the DNA solution is ≥50 μL.
4. Mix well and incubate for 5 minutes with periodic shaking (shaking is optional).
5. Bring magnet into contact with the tube(s) for 1 minute.

   **Fragments which are above the desired size range are bound to the sbeadex SAB and are discarded.**

6. Transfer the supernatant to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.
7. Add the corresponding volume of sbeadex SAB to the DNA solution based on the calculation shown in Figure 4.
8. Mix well and incubate for 5 minutes with periodic shaking (shaking is optional).
9. Bring magnet into contact with the tube(s) for 1 minute.
10. Remove the supernatant and discard.

   **Fragments of the desired size range are bound to the sbeadex SAB.**

11. Separate the magnet from the sample tubes.
12. Add 200 μL 75% ethanol (v/v in water). Mix thoroughly.
13. Incubate for 2 minutes with periodic shaking.
14. Bring magnet into contact with the tube(s) for 1 minute.
15. Remove the supernatant and discard.
17. Dry bead pellet in open tube for 5 minutes.
18. Add ≥30 μL Elution Buffer PN to the pellet. Mix thoroughly.
19. Incubate for 5 minutes with periodic shaking.
20. Bring magnet into contact with the tube(s) for 1 minute.
21. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.

**3.7 Dual sided size selection - purify DNA within a specific size range**

If a specific range of DNA fragment sizes are to be purified (e.g. all DNA fragments within a desired size range), the protocol requires an initial upper cut-off to exclude fragments that are too large, and a subsequent lower cut-off to exclude fragments that are too small.

The upper cut-off is performed first, with fragments that are too large being bound to the beads. The supernatant is retained, containing all fragments below the specified upper cut-off size, and is used as starting solution for the second lower cut-off. The subsequent lower cut-off binds all fragments greater than the specified size to the beads whilst smaller fragments remain in the supernatant and are discarded. At this point, fragments within the desired range are bound to the beads and the standard washing and elution steps can be performed.
The volume of sbeadex SAB required for the second binding (that is to perform the lower cut-off) is calculated using the formula below.

\[
[\text{Volume of SAB required for second binding}] = [\text{Desired bead ratio for second binding x starting DNA volume}] - [\text{Volume of SAB used in first binding}]
\]

Figure 7: Calculation for the volume of sbeadex SAB required to perform the lower cut-off for dual sided size selection for target peak sizes not outlined in Table 6.

For guidance on volumes of sbeadex SAB for dual side size selection, please refer to Table 6.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target peak size (bp)</td>
<td>Initial bead:sample ratio used (Step 3)</td>
<td>Additional bead:sample ratio added (Step 7)</td>
</tr>
<tr>
<td>250</td>
<td>0.65x</td>
<td>0.30x</td>
</tr>
<tr>
<td>300</td>
<td>0.60x</td>
<td>0.30x</td>
</tr>
<tr>
<td>350</td>
<td>0.58x</td>
<td>0.25x</td>
</tr>
<tr>
<td>400</td>
<td>0.55x</td>
<td>0.23x</td>
</tr>
<tr>
<td>450</td>
<td>0.53x</td>
<td>0.20x</td>
</tr>
<tr>
<td>500</td>
<td>0.50x</td>
<td>0.20x</td>
</tr>
<tr>
<td>550</td>
<td>0.49x</td>
<td>0.16x</td>
</tr>
<tr>
<td>600</td>
<td>0.48x</td>
<td>0.15x</td>
</tr>
</tbody>
</table>

Table 6: Recommended dual sided size selection sbeadex SAB/DNA volume ratios to be used below in section 3.7. Column A lists the desired target peak size for the dual sided size selected DNA sample. Column B illustrates the initial ratio of sbeadex SAB/DNA for right side size selection (Step 3 below). Column C illustrates the additional ratio of beads added to the right side size selected sample (Step 6 below) based on the original starting DNA-volume input required for left side size selection (Step 7 below). Together, these right and left size selection ratios will produce dual size selected samples with peak sizes listed. If you have different dual size selection targets, please use empirical testing based on figures 3, 5 and 7 to calculate the appropriate ratios for the right and left size selection steps.

Figure 8: Schematic of dual sided size selection protocol.
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sbeadex SAB (sequencing application beads)

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**Specific example of dual sided size selection:** Selection of DNA fragments with a peak size of 350 bp. This will require a 0.58x right side size selection followed by an added ratio of 0.25x for final of 0.83x for left side size selection.

i) The right side size selection is performed: 50 μL of starting DNA is combined with 29 μL sbeadex SAB (0.58x of DNA sample volume).

ii) The supernatant (79 μL) is transferred into a new tube.

iii) The left side size selection is performed: Determine the volume of buffer required for the second binding using Table 6. In this example, 12.5 μL of sbeadex SAB is added to the supernatant.

iv) The sbeadex SAB have now bound DNA fragments of with a peak size of 350 bp, and the supernatant can be removed.

v) Washing of the sbeadex SAB and elution steps can then be performed.

1. Bring beads to room temperature while protecting from light.
2. Thoroughly mix sbeadex SAB to ensure that beads are fully resuspended.
3. Based on the desired peak fragment size (Table 6, Column A), add the corresponding volume (appropriate ratio in Column B, Table 6 x starting DNA volume) of sbeadex SAB to the DNA solution. Ensure starting volume of DNA is ≥50 μL.
4. Mix well and incubate for 5 minutes with periodic shaking (shaking is optional).
5. Bring magnet into contact with the tube(s) for 1 minute.

   **Fragments which are above the desired size range are bound to the sbeadex SAB and are discarded.**

6. Transfer the supernatant to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.
7. Based on the desired peak fragment size (Table 6, Column A), add the corresponding volume (appropriate ratio from Table 6, Column C x original DNA starting volume) of sbeadex SAB to the DNA solution. Ensure total volume of DNA ≥50 μL.
8. Mix well and incubate for 5 minutes with periodic shaking (shaking is optional).
9. Bring magnet into contact with the tube(s) for 1 minute.

   **Fragments which are below the desired size range are remaining in the supernatant.**

10. Remove the supernatant and discard.

   **Fragments of the desired size range are bound to the sbeadex SAB.**

11. Separate the magnet from the sample tubes.
12. Add 200 μL 75% ethanol (v/v in water). Mix thoroughly.
13. Incubate for 2 minutes with periodic shaking.
14. Bring magnet into contact with the tube(s) for 1 minute.
15. Remove the supernatant and discard.
17. Dry bead pellet in open tube for 5 minutes.
18. Add ≥30 μL Elution Buffer PN to the pellet. Mix thoroughly.
19. Incubate for 5 minutes with periodic shaking.
20. Bring magnet into contact with the tube(s) for 1 minute.
21. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any sbeadex SAB.
4. Automating the sbeadex SAB kit protocol

Once the sbeadex SAB protocol has been tested (and optimised where necessary) manually for your sample type, it is possible to automate the procedure to increase throughput. Biosearch Technologies recommends following the manual protocol with respect to the volumes of buffers to use when automating the protocol. If you would like to discuss options for automation in your laboratory, please do not hesitate to contact our extractions specialists at Biosearch Technologies (see Section 7). We are able to offer pilot studies and customised protocols where required.

4.1. Mixing of samples

To mix samples efficiently using an automated liquid handling system, Biosearch Technologies recommends the following:

a) Set the mixing volume to between 50% and 80% of the volume to be mixed (instrument dependent).
b) For each mixing step, aspirate and dispense between 5 and 10 times (dependent on efficiency of the liquid handler).
c) Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete resuspension.

4.2. Automation via KingFisher

For automation on a KingFisher system (or similar) we recommend the following:

a) Keep all volumes the same as for manual sbeadex SAB kit, except for the elution volume. Due to evaporation on the KingFisher unit, 20 μL additional Elution Buffer PN should be added to the elution plate.
b) The incubation for each bind and wash step should be a minimum of 5 minutes to account for diffusion-dependent wash effects.
c) Prior to mixing for the binding, washing and elution steps, use the ‘Release Beads’ function with a ‘bottom mix’ for 10 seconds. Automated mixing should then be performed using the ‘Fast’ setting.

5. Troubleshooting

If issues are observed with the sbeadex SAB kit, please refer to Section 5.1 for Common troubleshooting solutions and section 5.2 for Frequently asked questions (FAQs). Alternatively, please contact our nucleic acid specialists, contact details in Section 7, who will be happy to assist you.
5.1 Common troubleshooting solutions to low yield/DNA recovery problems

<table>
<thead>
<tr>
<th>Low yield/DNA recovery problems</th>
<th>Possible solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement of recovered size-excluded products by spectrophotometric absorbance</td>
<td>Pre-size exclusion will most likely contain primers and nucleotides which will contribute to the absorbance, making the pre-size selected product appear more concentrated than the post-size excluded products. It is recommended to run the size-excluded products on a Bioanalyzer to assess the size of product recovered and its abundance.</td>
</tr>
<tr>
<td>Primer/adapter carryover</td>
<td>The ratio of sbeadex SAB volume to the input DNA volume determines the size of DNA fragments that bind to the sbeadex beads. During reaction clean-up, DNA of ~100 bp and greater is recovered, whilst removing adapters and primers of ~50 bp or less. Larger primers and adapters, or adapter dimers, may bind to the beads. If carryover of primer/adapter is an issue, the ratio of sbeadex SAB used can be decreased to lessen the likelihood of primer/adapter carryover. A second round of reaction clean-up can also be performed to further reduce primer/adapter carryover.</td>
</tr>
<tr>
<td>Magnetic bead loss</td>
<td>The nucleic acid will be bound to these beads, so any loss of beads during binding, washing or eluting will result in a loss of recovered nucleic acid. If beads are aspirated accidentally, return the supernatant back to the well, and reapply the magnet, or re-centrifuge. Then attempt to aspirate again.</td>
</tr>
<tr>
<td>Inaccurate pipetting</td>
<td>The sbeadex SAB kit will select desired DNA fragments dependent on the ratios of the volume of the sbeadex SAB to the volume of DNA. Therefore care should be taken when pipetting to ensure the appropriate volumes are being handled for the desired DNA fragment sizes to be selected. Increasing the initial sample volume can mitigate the effect of pipetting inaccuracy. We recommend a minimum DNA input volume of at least 50 μL, but this can be increased to 100 μL or more.</td>
</tr>
<tr>
<td>Insufficient mixing</td>
<td>It is critical to ensure that the beads are thoroughly mixed during the washing and elution stages of the protocol, and that the beads are thoroughly resuspended during the binding and elution stage.</td>
</tr>
<tr>
<td>Incorrect sbeadex kit used</td>
<td>Biosearch Technologies produces a variety of sbeadex-based kits for different functions. Ensure you are using the correct beads from the sbeadex SAB kit, and not magnetic beads from a sbeadex Extraction Kit.</td>
</tr>
</tbody>
</table>

Table 7: Troubleshooting low yield/DNA recovery issues.

5.2 Frequently asked questions (FAQs)

<table>
<thead>
<tr>
<th>Frequently asked question</th>
<th>Possible solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemistry and technology</td>
<td></td>
</tr>
<tr>
<td>How does the sbeadex SAB kit work?</td>
<td>The sbeadex SAB kit works on the principles of the concentration of the polyethylene-glycol (PEG) and salt in the buffer allowing the DNA to be precipitated onto the beads. Altering the concentration of the buffer will allow for different sized DNA fragments to bind to the beads.</td>
</tr>
<tr>
<td>Can the sbeadex SAB kit be used to size select both DNA and cDNA fragments?</td>
<td>The sbeadex SAB kit works on the technology that allows both DNA/cDNA to bind to the sbeadex SAB.</td>
</tr>
<tr>
<td>Is there a recommended extraction protocol for isolating DNA for next-generation sequencing (NGS) and sbeadex SAB?</td>
<td>We at Biosearch Technologies recommend using a Biosearch Technologies sbeadex Extraction Kit for isolation of NGS-quality nucleic acid. Other commercial extraction technology is available, but it should generate DNA which meets the minimum quantity/quality requirements of the NGS workflow.</td>
</tr>
</tbody>
</table>
Frequently asked question | Possible solution
--- | ---
Can the Elution Buffer AMP from the sbeadex Extraction Kits be used in substitution with the Elution Buffer PN from the sbeadex SAB kits? | It is not recommended to substitute the Elution Buffer PN with Elution Buffer AMP. Each reagent can be purchased individually from Biosearch Technologies.

Will the use of GC-rich or AT-rich DNA sequences introduce any bias in the sbeadex SAB kit? | The sequence of the DNA fragments is irrelevant to the binding efficiency of the sbeadex SAB.

Reagents and storage

The beads were accidently frozen. Will the performance of the beads be altered? | The performance of the beads has been assessed on the specified storage instructions. The performance of the beads cannot be guaranteed with any deviation from the stated storage conditions.

Once the DNA from the sbeadex SAB has been eluted, can the beads be reused? | It is not recommended to reuse the sbeadex SAB, due to the increased risk of DNA carry-over from one sample to the next. Use fresh sbeadex SAB for each sample.

Protocol optimisation

What adaptations can I make to the recommended protocol to increase the my final DNA recovery? | Ensure that your starting DNA is suspended in water, Tris or TE. Binding time of the DNA to the sbeadex SAB can be increased to 10 minutes. Also, the elution stage can be performed at higher temperatures, up to 60 °C.

I am getting carry over of different sized fragments in my final eluate. How can I minimise this? | To minimise the amount of undesired fragments being carried over into the elution stage, ensure exact volume are being pipetted for each reagent at each stage. This is particularly critical with the sbeadex SAB, as the amount of buffer used will directly affect the size of DNA fragments bound to the beads.

What is the recommended method for assessing the quantity and quality of the input and output DNA? | The starting DNA volume should be ≥50 μL. The quality of the DNA should be assessed using A260/280 and A260/230 measurements, and sizing of fragments can be carried out using electropherogram analysis.

After shearing, the DNA sample is very viscous and the final DNA recovery is very poor. What is causing this? | Very high starting DNA concentrations can cause the DNA sample to become viscous. This increased viscosity will interfere with the binding, and therefore the subsequent pelleting of the sbeadex SAB, affecting the final DNA recovery. If necessary, dilute the starting DNA with nuclease-free, molecular grade water.

6. Safety information

- Wear appropriate skin and eye protection throughout the preparation procedure
- Ensure kit components are stored appropriately according to local safety guidance
- In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Spillages can be removed using standard laboratory cleaning procedures
- Safety data sheets are available for all kit components upon request.

7. Further support

If you require any further support for the sbeadex SAB kit, please do not hesitate to contact our team of nucleic acid specialists:
Email: techsupport@lgcgroup.com
Integrated tools. Accelerated science.

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