Manual

sbeadex blood kit

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Not for use in diagnostic procedures.

BIOSEARCH™ TECHNOLOGIES

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Contents

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1. Introduction

sbeadex™ blood kits use magnetic separation for the purification of nucleic acids from 200 µL blood samples, including EDTA, heparin and citrate anticoagulation preparations. Superparamagnetic particles coated with sbeadex surface chemistry are used to capture nucleic acids from the blood sample, and utilises a novel two step binding mechanism which, when combined with the washing steps, removes impurities present in the sample matrix. After washing the nucleic acid is eluted and is ready for use in downstream processes. This kit is intended for research use only. It is not intended for use in diagnostic procedures.

2. Kit contents and storage conditions

Cat no.	Geographical region	Number of purifications per kit
NAP44401	RoW (excl. APAC)	96
NAP44404	APAC	96
NAP44410	RoW (excl. APAC)	960
NAP44440	APAC	960
NAP44100	RoW (excl. APAC)	5,000
NAP44400	APAC	5,000

Table 1: Catalogue numbers for sbeadex blood kits. RoW = Rest of the world; APAC = Asia-Pacific
*The number of purifications is based on LGC's standard protocol that assumes 200 µL starting blood volume.
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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.

Component	Colour	Storage conditions
Lysis buffer SB	Blue	Room temperature
Protease solution	Grey	Room temperature
Binding buffer SB	Green	Room temperature
sbeadex particles suspension + EDTA	White	Room temperature; 4 °C after opening
Wash buffer BN1	Red	Room temperature
Wash buffer TN1	Red	Room temperature
Wash buffer TN2	Yellow	Room temperature; 4 °C after opening
Elution buffer AMP	Black	Room temperature; 4 °C after opening

Table 2: sbeadex blood kit components and storage conditions.

Table 3 details the components supplied in each sbeadex blood kit.

Component	NAP44401 (96 preparation)	NAP44404 No Dangerous Goods (96 preparation)	NAP44410 (960 preparation)	NAP44440 No Dangerous Goods (960 preparations)	NAP44100 (5000 preparation)	NAP44400 No Dangerous Goods (5000 preparation)
Lysis Buffer SB	30 mL	30 mL	200 mL	200 mL	1200 mL	1200 mL
Binding Buffer SB	60 mL	-	500 mL	-	2000 mL	-
Binding Buffer SB (concentrate)	-	15 mL	-	125 mL	-	500 mL
Wash Buffer BN1	100 mL	-	1000 mL	-	4400 mL	-
Wash Buffer BN1 (concentrate)	-	67 mL	-	670 mL	-	2950 mL
Wash Buffer TN1	100 mL	-	1000 mL	-	4400 mL	-
Wash Buffer TN1 (concentrate)	-	67 mL	-	670 mL	-	2950 mL
Wash Buffer TN2	100 mL	100 mL	1000 mL	1000 mL	4400 mL	4400 mL
Elution Buffer AMP	30 mL	30 mL	200 mL	200 mL	1200 mL	1200 mL
Proteinase K	4 mL	4 mL	40 mL	40 mL	220 mL	220 mL
sbeadex Particle Suspension	4 mL	4 mL	40 mL	40 mL	220 mL	220 mL

Table 3: Components supplied in the sbeadex Blood DNA Kit, including details of component volumes by product code. The standard kits include ready-to-use buffers, whereas the no DG (non-dangerous goods) buffers must be prepared by adding specific alcohols, as indicated on the bottle labels.

3. Experimental procedure

3.1 General information before starting

When performing the sbeadex blood 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: 2000 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), unless otherwise stated.

It is important to ensure that the sbeadex particles suspension are properly resuspended before adding to the Binding buffer SB. Using non-homogenous sbeadex beads will affect the efficiency of the purification chemistry, potentially resulting in lower yields.

For information on automation of the sbeadex blood kit protocol, see Section 4 of this manual.

The following technical definitions and considerations (Table 4) should be applied to the experimental process, each time they are stated:

Technical description	Consideration
Bring magnet into contact with tubes	This will allow the sbeadex beads to form a pellet on the side of the tube, to allow for easy removal of the supernatant. The times stated for sbeadex bead pelleting are minimum recommended incubation times. The strength of the magnet will influence the speed of sbeadex beads pelleting, and so an increased incubation time should be used, if required, to ensure all beads are pelleted.
Mix thoroughly	The sample should be mixed thoroughly (preferably using a shaker), to ensure the sbeadex beads are completely resuspended. The mixing can be assisted by periodic vortexing in 5-10 second bursts.
Removal of supernatant	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.
Constant shaking	The sample should be constantly agitated by vortexing/shaking to ensure the sbeadex beads do not settle. This will increase the efficiency of the binding washing.

Table 4: Technical descriptions on 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) Magnetic rack or centrifuge
b) 96- or 384-well plates, or reaction tubes
c) RNase (optional – see Section 3.3)
d) Waterbath or incubator (for temperatures up to 55 °C)

3.3 Initial preparations

a) *Presence of precipitates:* Salt precipitates can form in the buffers at low temperatures. Check for the presence of precipitates prior to use, and if required, incubate buffers at 37 °C to until the precipitates have re-dissolved.

b) *Digestion with RNase (optional):* sbeadex beads co-isolate RNA during the purification, and RNA has been shown to influence downstream measurement chemistries or enzymatic processing. If RNA is shown to be affecting downstream processes, it is recommended to add 600 U RNase per 100 mL Wash buffer TN1.

c) *sbeadex particles suspension:* Mix the sbeadex beads thoroughly before use to fully resuspend the particles, before adding to the appropriate volume of Binding buffer SB. Each reaction requires 1.6x volume Binding buffer SB + 0.2x volume sbeadex particles suspension. Please refer to Table 5 for defined volumes per reaction for Binding buffer SB and sbeadex particles suspension for 100 µL and 200 µL starting blood volumes.

Kit component	Volume required	Volume for 100 µL starting blood volume (per reaction)	Volume for 200 µL starting blood volume (per reaction)
Binding buffer SB	1.6x	160 µL	320 µL
sbeadex particles suspension	0.2x	20 µL	40 µL
Total volume	**1.8x**	**180 µL**	**360 µL**

Table 5: Volumes of Binding buffer SB and sbeadex particles suspension for 100 µL and 200 µL starting blood volumes.

The following section outlines protocols for 100 µL and 200 µL starting blood volumes. Please refer to Table 6 for defined volumes of reagents for both 100 µL and 200 µL starting blood volumes.

Kit component	Volume required	Volume for 100 µL starting blood volume (per reaction)	Volume for 200 µL starting blood volume (per reaction)
Lysis buffer SB	1x	100 µL	200 µL
Protease solution	0.2x	20 µL	40 µL
Binding buffer SB with sbeadex particles suspension (see Table 3)	1.8x	180 µL	360 µL
Wash buffer BN1	4x	400 µL	800 µL
Wash buffer TN1	4x	400 µL	800 µL
Wash buffer TN2	4x	400 µL	800 µL
Elution buffer AMP	0.25x-1x	25-100 µL	50-200 µL

Table 6: Volumes of each component required for sbeadex blood kit protocol, for 100 µL and 200 µL starting blood volumes.

3.4 Manual sbeadex blood kit protocol

1. Add 1x volume Lysis Buffer SB to 1x volume blood, followed by 0.2x volume (of the input blood volume) Protease solution.
2. Incubate at 55 °C for 20 minutes, with constant shaking.
3. Let the samples cool to room temperature.
4. Add 1.8x volume of pre-mixed Binding buffer SB with sbeadex particles suspension (see Section 3.3 for preparation instructions).
5. Mix thoroughly and incubate for 5 minutes, with constant shaking.
6. Bring magnet into contact with the tube(s) for 2 minutes.
7. Remove the supernatant and discard.
8. Separate the magnet from the sample tubes.
9. Add 4x volume Wash buffer BN1. Mix thoroughly.
10. Incubate for 5-10 minutes, with periodic shaking.
11. Bring magnet into contact with the tube(s) for 2 minutes.
12. Remove the supernatant and discard.
13. Separate the magnet from the sample tubes.
14. Repeat steps 9-13 with Wash buffer TN1. *(Optional: add RNase – see Section 3.3).*
15. Repeat steps 9-13 with Wash buffer TN2.
16. Add 0.25x-1x volume Elution buffer AMP. Mix thoroughly.
17. Incubate for 10 minutes, with periodic shaking. *(Recommended: Incubate at temperatures up to 55 °C).*
18. Bring magnet into contact with the tube(s) for 3 minutes.
19. Transfer the eluate to a new tube by pipetting, avoiding the transfer of any sbeadex beads.

4. Automating the sbeadex blood kit protocol on the KingFisher™ Flex instrument

Once the sbeadex blood kit protocol has been trialled (and optimised where necessary) for your sample type manually, it is possible to automate the procedure to increase throughput. LGC 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 extraction specialists at LGC (see Section 7). Optimised protocols for running 100 µL and 200 µL blood volumes on the ThermoFisher™ KingFisher Flex 96 are available. Please contact our Technical Support Team (see Section 7) for further guidance.

To mix samples efficiently using an automated liquid handling system, LGC 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.

5. Troubleshooting

If issues are being observed with the sbeadex blood 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

Problem	Possible cause	Possible solution
PCR inhibition	Incomplete buffer removal	Ensure all buffer is completely removed before adding the next buffer.
	Incomplete lysis	Contact our Technical Support Team for further assistance.
Low yield	Inefficient binding	Ensure that the lysate, Binding buffer SB and sbeadex beads are mixed thoroughly.
	Excess of proteins limiting the DNA binding to the sbeadex beads	Follow the *Optional* steps outlined in the protocol.
	Incomplete lysis	Contact our Technical Support Team for further assistance.
Coloured eluates	Incomplete buffer removal	Ensure all buffer is completely removed before adding the next buffer.
	Incomplete lysis	Contact our Technical Support Team for further assistance.
Particles present in eluate	Aspirating too fast	Reduce the speed at which supernatants are removed.
	Loose pellet	Increase magnetic separation time/ centrifugation time to allow a tighter pellet to form.
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants.

Table 7: Common troubleshooting solutions.

5.2. Frequently asked questions (FAQs)

Frequently asked question	Possible solution
Can I use coagulated blood with the sbeadex blood kit?	Yes, however longer lysis times may be required in order to address possible inhibition. Contact our Technical Support Team (see Section 7) for further guidance.
Is there a specific order of adding the Lysis buffer SB and the Protease solution to the sample?	Yes, it is recommended to add the components to the reaction tube in either; a) Protease solution then blood sample then Lysis buffer SB or, b) Lysis buffer SB then blood sample then Protease solution. Adding Protease solution directly to Lysis buffer SB may inactivate the activity of the Protease solution.
Can I use both a waterbath and an incubator for any heat steps?	Yes, both pieces of equipment are suitable. However, it should be noted that heat conduction occurs more efficiently in a waterbath compared with an incubator. Therefore incubation times may have to be adjusted depending on the equipment used. Please contact our Technical Support Team for further advice.

Table 8: Frequently asked questions (FAQs).

6. Safety information

- Wear appropriate skin and eye protection throughout the preparation procedure
- Lysis buffer SB, Binding buffer SB and Wash buffer TN1 contain high concentrations of detergent and salt
- Binding buffer SB and Wash buffer TN1 contain up to 50% n-propanol, therefore keep away from naked flames
- 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 on request

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer SB	 Warning	H302/H315/H319/H400	P101/P102/P103/P273/ P280/P305+P351+P338/ P301+P312/P332+P313/P501 /P301+P312
Protease solution	Danger 	H334/H317	P101/P102/P103/P261/ P304+P341/P501
Binding buffer SB	 Danger	H226/H302/H315/H318/H336/ H400	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310/P501
sbeadex particles suspension	-	-	-
Wash buffer BN1	 Danger	H226/H332/H315/H318/H336	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310/ P405/P501
Wash buffer TN1	 Danger	H315/H318/H226/H336	P101/P102/P103/P210/ P303+P361+P353/ P305+P351+P338/P310/P405/ P501
Wash buffer TN2	-	-	-
Elution buffer AMP	-	-	-

Table 9: Safety information for sbeadex blood kit components.

7. Further support

If you require any further support for the sbeadex blood kit, please do not hesitate to contact our Technical Support Team: **techsupport@lgcgroup.com**

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