Manual

sbeadex Pathogen Nucleic Acid Purification Kit

NAP40-024-01/...-02/...-03/...-04
NAP40-025-01/...-02/...-03/...-04
NAP40-026-01/...-02/...-03/...-04
NAP40-027-01/...-02/...-03/...-04

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BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

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1. Introduction

sbeadex™ Pathogen Nucleic Acid Purification Kit from LGC Biosearch Technologies™ uses magnetic separation for the purification of viral and bacterial RNA and DNA from nasopharyngeal swab and sputum samples. A list of pathogen species and compatible matrices can be found on our website.

Superparamagnetic particles coated with sbeadex surface chemistry use a novel two-step binding mechanism which, when combined with the washing steps, removes impurities present in the sample matrix. After washing, the nucleic acids are eluted and then ready for use in downstream PCR and real-time PCR applications.

This protocol has been verified for a range of sample matrices. These are sample swabs in viral transport medium (VTM), sputum, whole blood, serum, plasma, urine, stool and cerebrospinal fluid. Sputum was prepared following CDC guidelines. Additional matrices may be compatible with the sbeadex Pathogen Nucleic Acid Purification Kit but may require additional protocol optimisation.

This kit is validated for research use only. It is not intended for use in diagnostic procedures.

Figure 1. Easy 6-step purification procedure using Biosearch Technologies' innovative sbeadex technology.

2. Kit contents and storage conditions

All kit components should be used by the expiry date stated on the kit box, and stored under the recommended storage conditions.

Component	Colour	Storage conditions
Lysis buffer SB	Blue	Room temperature
Protease solution*	Grey	Room temperature; 4 °C after opening
Binding buffer SB**	Green	Room temperature
sbeadex particle 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

*Protease solution is only included in kits with part codes NAP40-024-XX and NAP40-025-XX.
**Please note that Asia-Pacific (APAC)-suitable kits (part codes NAP40-025-XX and NAP40-027-XX) do not contain n-propanol in Binding buffer SB, and Wash Buffers BN1 and TN1; refer to the bottle labels for instructions on how much n-propanol to add before commencing the protocol.

Table 1. sbeadex Pathogen Nucleic Acid Purification Kit components and storage conditions

3. Experimental procedure

3.1. General information before starting

When performing the sbeadex Pathogen Nucleic Acid Purification Kit protocol, a magnetic rack or centrifuge is required to pellet the magnetic particles.

If performing the protocol manually without access to a magnetic rack, sample tubes can be centrifuged for 10 seconds (single tubes at full speed; plates at 2000 x g) to enable the magnetic particles to form a pellet.

All processes should be carried out at room temperature (15-25 °C) unless otherwise stated.

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

The considerations in Table 2 should be applied to the experimental process, each time the specific protocol process is stated in the step-by-step method:

Protocol process	
Bring magnetic rack 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 magnetic rack will influence the speed of sbeadex beads pelleting. If required, increasing incubation time should be used 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. To avoid disruption of the particle pellet when placing the pipette tip inside the tube, ensure that the tip is aimed towards the sample tube wall opposite the 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 movement will increase the efficiency of the binding and washing steps.

Table 2. Technical descriptions of processes required in this protocol, and considerations that should be adhered to when performing these steps

3.2. Required materials (not included)

a. Magnetic rack or centrifuge
b. 96- or 384-well plates or reaction tubes that are RNase-free
c. Water bath or incubator (for temperatures up to 55 °C)
d. **Optional:** DNA/RNA carrier. Carrier DNA/RNA may improve sensitivity if pathogens are in low concentrations in background genetic material.
e. **Optional:** 1X PBS (molecular biology grade). Required for preparation of stool and sputum samples.
f. **Optional:** Protease solution (only included in kits with part codes NAP40-024-XX and NAP40-025-XX). Proteinase digestion may be required for lysis of some bacterial species, or for liquifying the sample matrix.

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 until the precipitates have re-dissolved.
b. Preparing the sbeadex particle suspension: The sbeadex particle suspension and Binding buffer SB can be added to the reaction(s) as a premix.
   To prepare the premix for the sbeadex RNA purification protocol:
   i. Thoroughly mix the sbeadex particle suspension to fully resuspend the particles;

ii. Add 20 µL sbeadex particle suspension to 160 µL Binding buffer SB;
iii. If preparing premix for multiple reactions, multiply the volumes accordingly and allow sufficient overage for accurate pipetting.

c. APAC-suitable kits only: For part codes NAP40-025-XX and NAP40-027-XX, please ensure that the appropriate volumes of n-propanol are added to Binding buffer SB, Wash buffer BN1, and Wash buffer TN1 prior to commencing the protocol. Refer to the individual bottle labels for details.

3.4. Stool sample preparation

Stool (or faecal) samples require dilution before they are used in a purification reaction. Biosearch Technologies suggests using a spatula of approximately 5 mm diameter to transfer a sub-sample of stool into a 1.5 mL reaction tube. Dilute stool in 1 mL of 1X PBS (per spatula of sample), vortex vigorously and spin down the remaining solid fragments. Transfer the supernatant to a fresh tube and use 100 µL of this diluted sample material in each purification reaction. Do not carry over any solid material into the sbeadex reaction.

3.5 Lysis recommendations for different sample matrices

The optimal duration of the lysis step depends on the pathogen and the sample matrix of interest. Biosearch Technologies has the following recommendations, although you may wish to perform optimisation of the lysis step in your laboratory to determine the most efficient timings for your specific samples:

a. **Whole blood, plasma, serum, urine and cerebrospinal fluid:** A lysis time of three minutes was found to be optimal. Please note that for whole blood, prolonged lysis (beyond three minutes) could lead to pathogen RNA degradation.
b. **Stool samples:** A lysis time of 10 minutes is recommended.

Please note that some combinations of pathogen and matrix may not require a lysis step at all. For these samples, the lysis and binding step can be performed simultaneously. For this, prepare a ‘binding mix’ containing lysis buffer, binding buffer and beads, and add this ‘binding mix’ to the sample. Continue with the protocol as stated in Table 3 and Table 4 from the column entitled “Binding”. Some sample types may benefit from a longer lysis step (up to 20 minutes) and/or a pre-lysis step (see Section 3.6.).

3.6. Optional pre-lysis for bacterial samples

1. Add the following to the reaction tube/well in the order listed below:
   a. **Optional:** 20 µL Protease solution
   b. **Optional:** 1 µg carrier DNA/RNA
   c. 100 µL of the liquid starting sample
   d. 100 µL (1x) Lysis buffer SB.
2. Incubate at 55 °C for 10 minutes with constant shaking
3. Allow the sample(s) to cool to room temperature
4. Proceed to the step-by-step protocol (Section 3.8.).

NOTE: Some bacterial species may require further treatment (i.e. heat inactivation at 90 °C and/or zirconium beads) to disrupt the cell wall.

3.7. Overview of the manual nucleic acid purification protocol

Table 3 below summarises the standard manual sbeadex Pathogen Nucleic Acid Purification Kit protocol, including volumes of each component and the time and temperature for each step.

STEP	Lysis				Binding	Wash (x3)	Elution
COMPONENT	**Optional*:** Protease solution (20 µL)	**Optional**:** Carrier DNA/RNA (e.g. 1 µg PolyA)	Sample (100 µL)	Lysis buffer SB (100 µL)	Binding buffer SB (160 µL) + sbeadex particle suspension (20 µL)	Wash buffers: 1. BN1 (400 µL) 2. TN1 (400 µL) 3. TN2 (400 µL)	Elution buffer AMP (100 µL)
CONDITION				***0-20 min 55 °C	10 min Room temperature	5 min Room temperature	10 min 60 °C

*Optional: Proteinase digestion may be required for lysis of some bacterial species, or for liquifying the sample matrix.
**Optional: Carrier DNA/RNA may improve sensitivity if pathogens are in low concentrations in background genetic material.
***If Protease solution is not being used, the incubation step at 55 °C is not required.

Table 3. Summary of the standard manual sbeadex Pathogen Nucleic Acid Purification Kit protocol

3.8. Step-by-step manual protocol for nucleic acid purification post lysis

1. Add 20 µL sbeadex particle suspension and 160 µL Binding buffer SB (these can be added as a 180 µL premix – see section 3.3.).
2. Mix thoroughly and incubate for 5 minutes at room temperature, with constant shaking.
3. Bring magnetic rack into contact with the plate(s) or tube(s) for 2 minutes.
4. Remove the supernatant and discard.
5. Separate the magnetic rack from the sample plate(s) or tube(s).
6. Add 400 µL Wash buffer BN1.
7. Incubate for 5 minutes at room temperature, with constant shaking.
8. Bring magnetic rack into contact with the plate(s) or tube(s) for 2 minutes.
9. Remove the supernatant and discard.
10. Separate the magnetic rack from the sample plates(s) or tube(s).
11. Repeat steps 6-10 with Wash buffer TN1.
12. Repeat steps 6-10 with Wash buffer TN2.
13. Add 100 µL Elution buffer AMP. Mix thoroughly.
14. Incubate for 10 minutes at 60 °C with periodic shaking.
15. Bring magnetic rack into contact with the plate(s) or tube(s) for 3 minutes.
16. Transfer the nucleic acid-containing eluate to a new well or tube by pipetting, avoiding the transfer of any sbeadex beads.

4. Automating the nucleic acid purification protocol

After trialling the sbeadex Pathogen Nucleic Acid Purification Kit protocol for your sample type manually, and optimising where necessary, it is possible to automate the procedure to increase throughput. Biosearch Technologies have optimised the manual protocol for automation on the KingFisher Flex magnetic particle processor (ThermoFisher Scientific) for 100 µL starting volumes.

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

a. Set the mixing volume 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.

4.1. Automation on the KingFisher Flex

In addition to the components provided in the kit, the user needs to provide the following:

- Tips
- KingFisher deep-well plates (4 per extraction)
- KingFisher standard plates (4 per extraction)
- KingFisher comb (1 per extraction)
- Optional: Carrier DNA/RNA.

The recommended starting protocol for the KingFisher Flex has a total time of 22 minutes and is available together with three alternative protocols. These differ only in the lysis time which ranges from zero to 20 minutes. The BindIt (.bdz) files* for these protocols are available from Biosearch Technologies. The protocols are summarised in Table 4.

*Depending on the sample matrix, a .bdz file consisting of a longer protocol based on timings for the manual protocol is also available from Biosearch Technologies if required.

STEP	Lysis				Binding	Wash (x3)	Elution
COMPONENT	**Optional*:** Protease solution (20 µL)	**Optional**:** Carrier DNA/RNA (e.g. 1 µg PolyA)	Sample (100 µL)	Lysis buffer SB (100 µL)	Binding buffer SB (160 µL) + sbeadex particle suspension (20 µL)	Wash buffers: 1. BN1 (400 µL) 2. TN1 (400 µL) 3. TN2 (400 µL)	Elution buffer AMP (100 µL)
CONDITION				***0-20 min 55 °C	5 min Room temperature	1 min Room temperature	5 min 60 °C

*Optional: Proteinase digestion may be required for lysis of some bacterial species, or for liquifying the sample matrix.
**Optional: Carrier DNA/RNA may improve sensitivity if pathogens are in low concentrations in background genetic material.
***If Protease solution is not being used, the incubation step at 55 °C is not required.

Table 4. Summary of the KingFisher automated sbeadex Pathogen Nucleic Acid Purification Kit protocol

5. Troubleshooting

If issues are observed with the sbeadex Pathogen Nucleic Acid Purification Kit, please refer to section 5.1. for common troubleshooting solutions and section 5.2. for frequently asked questions (FAQs). Alternatively, please contact our technical support team; see section 7 for details.

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 in the procedure.
	Incomplete lysis	Contact our technical support team for assistance.
Low yield	RNA degradation before stabilised as cDNA	Store RNA at -80 °C. Use RNase free plastics.
	Sample is degraded	Store input sample at -80 °C prior to use.
	Inefficient binding	Ensure that the lysate, Binding buffer SB, and sbeadex beads are mixed thoroughly.
Particles present in eluate	Aspirating too fast	Reduce the speed at which supernatants are removed.
	Loose pellet	Increase magnetic separation or centrifugation time to allow formation of a tighter pellet.
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants.

Table 5. Common troubleshooting solutions for the sbeadex Pathogen Nucleic Acid Purification Kit

5.2. Frequently asked questions (FAQs)

Question	Possible solution
How do I safely inactivate biohazardous flow-through material?	Always dispose of potentially biohazardous solutions according to your institution's waste-disposal guidelines. Although the lysis and binding buffers in sbeadex kits contain chaotropic agents that can inactivate some biohazardous material, local regulations dictate the proper way to dispose of biohazards. **DO NOT** add bleach or acidic solutions directly to the sample-preparation waste. The guanidine hydrochloride present in the sample-preparation waste can form highly reactive compounds when combined with bleach. Please access our safety data sheet (SDS) online for detailed information on the reagents for each respective kit.
Can I use both a water bath 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 water bath compared to an incubator. Incubation times may therefore have to be adjusted depending on the equipment used. Please contact our technical support team for further advice.
What is the recommended method for assessing the quantity and quality of the purified viral RNA?	The recommended method for assessing the purified RNA is through real-time reverse transcription quantitative PCR (RT-qPCR).
Once the nucleic acid is eluted, can the beads be reused?	Do not reuse the sbeadex beads. There is risk of nucleic acid carryover from one sample to the next. Use fresh sbeadex beads for each sample.

Table 6. Frequently asked questions for the sbeadex Pathogen Nucleic Acid Purification Kit. If you have additional questions, please contact our technical support team (see section 7 for details).

6. Safety information

To access the SDS documents for the components in this kit, please visit our website. Work with infectious virus should be carried out according to the regulation of the country within which the kit is being used.

- 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.
- SDSs are available for all kit components on request.
- Always handle kit components in well ventilated areas.

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/ P241/P303+P361+P353/ P305+P351+P338/P310/P501
sbeadex particle 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 7. Safety information for sbeadex Pathogen Nucleic Acid Purification Kit components

7. Further support

If you require any further assistance with this kit, please contact our technical support team at techsupport@lgcgroup.com and we will be happy to help.

8. Ordering information

The following table details the sbeadex Pathogen Nucleic Acid Purification Kits available from Biosearch Technologies, including pack size and geographical region information.

Geographical region	Part code	Number of purifications per kit	Sample volume
RoW (excluding APAC)	**NAP40-024-01**	96 (trial kit)	100 µL
	NAP40-024-02	960	100 µL
	NAP40-024-03	5,000	100 µL
	NAP40-024-04	10,000	100 µL
RoW (excluding APAC) (no Protease solution)	**NAP40-026-01**	96 (trial kit)	100 µL
	NAP40-026-02	960	100 µL
	NAP40-026-03	5,000	100 µL
	NAP40-026-04	10,000	100 µL
APAC-suitable (without dangerous goods)	**NAP40-025-01**	96 (trial kit)	100 µL
	NAP40-025-02	960	100 µL
	NAP40-025-03	5,000	100 µL
	NAP40-025-04	10,000	100 µL
APAC-suitable (without dangerous goods, no Protease solution)	**NAP40-027-01**	96 (trial kit)	100 µL
	NAP40-027-02	960	100 µL
	NAP40-027-03	5,000	100 µL
	NAP40-027-04	10,000	100 µL

Table 8. Part codes for sbeadex Pathogen Nucleic Acid Purification Kits. RoW = rest of world. APAC = Asia Pacific. Number of purifications is based on a starting volume of 100 µL sample.

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