

Manual

sbeadex viral RNA purification kit

NAP-40-024-02 / ...-03 / ...-04

NAP-40-025-02 / ...-03 / ...-04

NAP-40-026-02 / ...-03 / ...-04

NAP-40-027-02 / ...-03 / ...-04

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Manual

sbeadex viral RNA purification kit

Contents

[1. Introduction](#)

[2. Kit contents and storage conditions](#)

[3. Experimental procedure](#)

[3.1 General information before starting](#)

[3.2 Required materials \(not included\)](#)

[3.3 Initial preparations](#)

[3.4 Overview of the RNA purification protocol](#)

[3.5 Step-by-step protocol for RNA purification](#)

[4. Automating the RNA purification protocol](#)

[4.1 Automation on the KingFisher Flex](#)

[5. Troubleshooting](#)

[5.1 Common troubleshooting solutions](#)

[5.2 Frequently asked questions \(FAQs\)](#)

[6. Safety information](#)

[7. Further support](#)

[8. Ordering information](#)

Manual

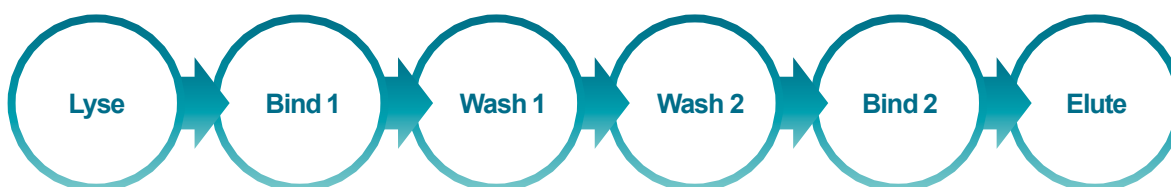
sbeadex viral RNA purification kit

1. Introduction

sbeadex viral RNA purification kit from LGC, Biosearch Technologies™ uses magnetic separation for the purification of viral RNA from nasopharyngeal swab and sputum samples. 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 RNA is eluted and is ready for use in downstream real-time PCR applications.

This protocol has been verified using sample swabs shaken in viral transport medium (VTM) or sputum samples, both prepared following CDC guidelines.

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



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	
Lysis buffer SB	Blue	Room temperature
Protease solution*	Grey	4 °C
Binding buffer SB**	Green	Room temperature
sbeadex particles suspension	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 NAP-40-024-XX and NAP-40-025-XX.

**Please note that Asia-Pacific (APAC)-suitable kits (part codes NAP-40-025-XX and NAP-40-027-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 viral RNA purification kit components and storage conditions.

Manual

sbeadex viral RNA purification kit

3. Experimental procedure

3.1 General information before starting

When performing the sbeadex RNA purification protocol, a magnetic rack or centrifuge is required to pellet the magnetic particles.

If performing the protocol manually without access to a magnetic racks, 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 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 particles 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 following considerations (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	Consideration
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.

Manual

sbeadex viral RNA purification kit

3.2 Required materials (not included)

- Magnetic rack or centrifuge
- 96- or 384-well plates or reaction tubes that are RNase-free
- Water bath or incubator (for temperatures up to 55 °C)
- Optional: DNA/RNA carrier. Biosearch Technologies has generated scientific data with AccuPlex™ Reference Material from LGC SeraCare Life Sciences showing that the use of Protease solution (Proteinase K) and carrier RNA was not required and has no influence on data quality.

3.3 Initial preparations

- 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.
- Preparing the sbeadex particles suspension: The sbeadex particles 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:
 - Thoroughly mix the sbeadex particle suspension to fully resuspend the particles;
 - Add 20 µL sbeadex particle suspension to 160 µL Binding buffer SB;
 - If preparing premix for multiple reactions, multiply the volumes accordingly and allow sufficient overage for accurate pipetting.
- APAC-suitable kits only: For part codes NAP-40-025-XX and NAP-40-027-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 Overview of the RNA purification protocol

Table 3 below summarises the standard manual sbeadex viral purification 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				**10 min **55 °C	10 min Room temp	5 min Room temp	10 min 60 °C

*Optional: Biosearch Technologies has generated scientific data with AccuPlex Reference Material from LGC SeraCare Life Sciences showing that the use of protease solution (Proteinase K) and carrier RNA was not required and has no influence on data quality.

**If Protease solution is not being used, the incubation step for 10 minutes at 55 °C is not required.

Table 3. Summary of the standard manual sbeadex viral RNA purification protocol.

Manual

sbeadex viral RNA purification kit

3.5 Step-by-step protocol for RNA purification

1. Add the following to the reaction tube in the order listed below:
 - a. **Optional*:** 20 µL Protease solution to the reaction tube/well
 - b. **Optional*:** 1 µg carrier DNA/RNA
 - c. 100 µL of the liquid starting sample
 - d. 100 µL (1x) Lysis buffer SB.

***Optional:** Biosearch Technologies has generated scientific data with AccuPlex Reference Material from LGC Seracare Life Sciences showing that the use of Protease solution and carrier RNA was not required and has no influence on data quality.
2. *Only if Protease solution is being used:* Incubate at 55 °C for 10 minutes with constant shaking.
3. *Only if Protease solution is being used:* Allow the sample(s) to cool to room temperature.
4. Add 20 µL sbeadex particles suspension and 160 µL Binding buffer SB (these can be added as a 180 µL of premix – see step 1.2 above).
5. Mix thoroughly and incubate for 5 minutes at room temperature with, constant shaking.
6. Bring magnetic rack into contact with the plate(s) or tube(s) for 2 minutes.
7. Remove the supernatant and discard.
8. Separate the magnetic rack from the sample plate(s) or tube(s).
9. Add 400 µL Wash buffer BN1.
10. Incubate for 5 minutes at room temperature, with constant shaking.
11. Bring magnetic rack into contact with the plate(s) or tube(s) for 2 minutes.
12. Remove the supernatant and discard.
13. Separate the magnetic rack from the sample plates(s) or tube(s).
14. Repeat steps 9-13 with Wash buffer TN1.
15. Repeat steps 9-13 with Wash buffer TN2.
16. Add 100 µL Elution buffer AMP. Mix thoroughly.
17. Incubate for 10 minutes at 60 °C with periodic shaking.
18. Bring magnetic rack into contact with the plate(s) or tube(s) for 3 minutes.
19. Transfer the eluate to a new well or tube by pipetting, avoiding the transfer of any sbeadex beads.

4. Automating the RNA purification protocol

After trialling the sbeadex viral RNA 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);

Manual

sbeadex viral RNA purification kit

- 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 optimised protocol for the KingFisher Flex has a total protocol time of 22 minutes. The [BindIt \(.bdz\) file](#) for this protocol is available from Biosearch Technologies. The protocol is 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				3 min 55 °C	5 min Room temp	1 min Room temp	5 min 60 °C

*Optional: Biosearch Technologies has generated scientific data with AccuPlex Reference Material from LGC SeraCare Life Sciences showing that the use of protease solution (Proteinase K) and carrier RNA was not required and has no influence on data quality.

Table 4. Summary of the KingFisher automated sbeadex viral RNA purification protocol.

Manual

sbeadex viral RNA purification kit

5. Troubleshooting

If issues are being observed with the sbeadex RNA 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.
	RNA degradation before stabilised as cDNA	Store RNA at -80 °C. Use RNase free plastics.
Low yield	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 viral RNA purification kit.

Manual

sbeadex viral RNA 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 viral RNA is through real-time reverse transcriptase quantitative PCR (RT-qPCR).
Once the RNA is eluted, can the beads be reused?	Do not reuse the sbeadex RNA beads. There is risk of RNA carryover from one sample to the next. Use fresh sbeadex RNA beads for each sample.

Table 6. Frequently asked questions for the sbeadex viral RNA 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.

Manual

sbeadex viral RNA purification kit






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 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 7. Safety information for sbeadex viral RNA purification kit components.

Manual

sbeadex viral RNA purification kit

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 viral RNA 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)	NAP-40-024-02	960 (trial kit)	100 µL
	NAP-40-024-03	5,000	100 µL
	NAP-40-024-04	10,000	100 µL
RoW (excluding APAC) (no Protease solution)	NAP-40-026-02	960 (trial kit)	100 µL
	NAP-40-026-03	5,000	100 µL
	NAP-40-026-04	10,000	100 µL
APAC-suitable (without dangerous goods)	NAP-40-025-02	960 (trial kit)	100 µL
	NAP-40-025-03	5,000	100 µL
	NAP-40-025-04	10,000	100 µL
APAC-suitable (without dangerous goods, no Protease solution)	NAP-40-027-02	960 (trial kit)	100 µL
	NAP-40-027-03	5,000	100 µL
	NAP-40-027-04	10,000	100 µL

Table 8. Part codes for sbeadex viral RNA 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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