Automation of sbeadex Pathogen Nucleic Acid Purification Kit using the KingFisher Flex

Protocol

For Research Use Only.
Not for use in diagnostic procedures.

1. Introduction

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. LGC, Biosearch Technologies™ has validated the sbeadex Pathogen Nucleic Acid Purification Kit using sample swabs in viral transport medium (VTM), sputum, whole blood, serum, plasma, urine, stool and cerebrospinal fluid. Sputum was prepared following CDC guidelines. The manual protocol is optimised for automation on the KingFisher™ Flex magnetic particle processor (ThermoFisher Scientific) for 100 µL starting volumes. Biosearch Technologies recommends following the manual protocol with respect to volumes of buffers (see Table 2) to use when automating the protocol.

2. Kit contents and customer requirements

Table 1 details the contents of the sbeadex Pathogen Nucleic Acid Purification Kit, and equipment that the user is responsible for providing for automation of the protocol on the KingFisher.

Included in the sbeadex Pathogen Nucleic Acid Purification Kit	Not included in the sbeadex Pathogen Nucleic Acid Purification Kit; customer to provide
Lysis buffer SB	Tips
Binding buffer SB	Pipette
Wash buffer BN1	4 x KingFisher deep-well plates (per extraction)
Wash buffer TN1	2 x KingFisher standard plates (per extraction)
Wash buffer TN2	1 x KingFisher combs (per extraction)
Elution buffer AMP	Optional: Carrier RNA/DNA
Protease solution*	
sbeadex particle suspension	
User guide/protocol	

* Protease solution is only included in kits with part codes NAP40-024-XX and NAP40-025-XX

Table 1: Kit contents and customer requirements for KingFisher automation of the sbeadex Pathogen Nucleic Acid Purification Kit.

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3. Manual protocol summary

Table 2 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 temp	5 min Room temp	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

Table 2. Summary of the standard manual sbeadex Pathogen Nucleic Acid Purification Kit protocol.

4. Optional pre-lysis for bacterial samples

For bacterial samples, pre-lysis before transferring to the KingFisher Flex may be required.

1. Add the following to the reaction 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 KingFisher automated protocol (Section 5).

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

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5. Optimised KingFisher Flex protocol

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 twenty minutes. The BindIt (.bdz) files* for these protocols are available from Biosearch Technologies. The protocols are summarised in Table 3.

*Certain sample matrices may require a longer protocol. Please contact our Techical Support Team (see Section 6) to request a .bdz file based on timings for the manual protocol.

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 temp	1 min Room temp	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.

Table 3. Summary of the KingFisher-automated sbeadex Pathogen Nucleic Acid Purification Kit protocol

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.

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Overview of plates on the KingFisher

Binding plate	KingFisher 96 deep-well plate	
Optional: Protease solution	20 µL	Reagent
Sample	100 µL	Reagent
Lysis buffer SB	100 µL	Reagent
Optional: Carrier DNA/RNA	1 µg	Reagent

Washing plate BN1	KingFisher 96 deep-well plate	
Wash buffer BN1	400 µL	Reagent

Washing plate TN1	KingFisher 96 deep-well plate	
Wash buffer TN1	400 µL	Reagent

Washing plate TN2	KingFisher 96 deep-well plate	
Wash buffer TN2	400 µL	Reagent

Elution plate	KingFisher standard 96-well plate	
Elution buffer AMP	100 µL	Reagent

Comb		
-	-	-

Dispensed reagents on the KingFisher

Binding plate	KingFisher 96 deep-well plate	
Binding buffer SB	Add Binding buffer SB and beads	160 µL
sbeadex particle suspension	Add Binding buffer SB and beads	20 µL

6. Further support

If you would like to discuss options for automation in your laboratory, or require any further guidance, please do not hesitate to contact our Technical Support Team at techsupport@lgcgroup.com.

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