Automation of sbeadex viral RNA purification kit using the KingFisher Flex

Protocol

1. Introduction

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. LGC, Biosearch Technologies has validated the sbeadex viral RNA purification kit using non-clinical material (swabs shaken in universal transport media (UTM) or sputum, both prepared following CDC guidelines), and have optimised the manual protocol 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 to use when automating the protocol.

2. Kit contents and customer requirements

Table 1 details the contents of the sbeadex viral RNA purification kit, and equipment that the user is responsible for providing for automation of the protocol on the KingFisher.

Included in the sbeadex viral RNA kit	Not included in the sbeadex viral RNA 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 BN2	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 NAP-40-024-XXX and NAP-40-025

Table 1: Kit contents and customer requirements for KingFisher automation of the sbeadex viral RNA purification kit.

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3. Manual protocol summary

Table 2 below summarises the standard manual sbeadex viral RNA 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 and carrier DAN/RNA was not required and has no influence on data quality.
**If Protease solution is not being used, the 10 minutes at 55 ºC incubation step is not required.

Table 2. Summary of the standard manual sbeadex viral RNA purification protocol.

4. Optimised KingFisher Flex protocol

The manual sbeadex viral RNA purification kit protocol has been optimised for automation on the KingFisher Flex, with 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 3.

*Depending on the sample matrix, a .bdz file consisting of a longer protocol based on timings for the manual protocol will also be 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 and carrier DNA/RNA was not required and has no influence on data quality.

Table 3. Summary of the KingFisher-automated sbeadex viral RNA purification 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.

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

Comb	KingFisher 96 deep-well plate	
Binding buffer SB	Add Binding buffer SB and beads	20 µL
sbeadex particle suspension	Add Binding buffer SB and beads	100 µL

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5. 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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