Automation of sbeadex Pathogen Nucleic Acid Purification Kit using the oKtopure

Protocol

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 non-clinical material (swabs shaken in universal transport media (UTM) or sputum prepared following CDC guidelines), and have optimised the manual protocol for automation on the oKtopure™ liquid handler 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 Pathogen Nucleic Acid Purification Kit, and equipment that the user is responsible for providing for automation of the protocol on the oKtopure.

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	96-well non-sterile tip rack (KBS-0010-003)
Binding buffer SB	Pipette
Wash buffer BN1	1.2 mL 96-well storage plate (KBS-7001-130)
Wash buffer TN1	0.8 mL 96-well storage plate (KBS-7001-131)
Wash buffer TN2	2 mL square well waste plates
Elution buffer AMP	Optional: Carrier RNA/DNA
Protease solution*	oKtowash (KBS-0009-002)
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 oKtopure 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 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: 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 for 10 minutes at 55 °C is not required.

Table 2. Summary of the standard manual sbeadex pathogen nucleic acid purification protocol.

4. Optimised oKtopure protocol

The manual sbeadex Pathogen Nucleic Acid Purification Kit protocol has been optimised for automation on the oKtopure, with a total protocol time of 150 minutes for 8 plates. The oKtopure script for this protocol is available from Biosearch Technologies. The protocol is summarised in Table 3.

STEP	Lysis offline				Binding	Wash (x3)	Elution
COMPONENT	**Optional:*** Protease solution* (5 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 (240 µL) 2. TN1 (150 µL) 3. TN2 (340 µL)	Elution buffer AMP (50 µL)
CONDITION				30 min 95 °C (in an oven)	5 min Room temp	5 min Room temp	5 min

* 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 oKtopure-automated sbeadex pathogen nucleic acid 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.

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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