

sbeadex® blood kit and KingFisher 96 instrument

F. Schubert, LGC Genomics, Ostendstrasse 25, TGS Haus 8, 12459 Berlin, Germany



Description

The sbeadex® blood kit (Cat. No. 41403 or 41430) has been developed for high throughput extraction of genomic DNA from whole blood and buffy coats. The magnetic particle-based DNA extraction protocol can be easily automated using a KingFisher 96 (Thermo Fisher Scientific) magnetic particle manipulator. Using magnetic rods, which are protected against contamination by a tip comb, the magnetic beads are transferred from one buffer plate to the next during the extraction process. This instrument can process up to 96 samples per run. In addition, the KingFisher 96 instrument is compatible with liquid handling systems and sample handling devices, thus making a hands-free medium to high throughput system a reality.

sbeadex coated magnetic particles bind DNA using a novel two-step binding mechanism in the presence of detergents and salts. After binding and washing steps, the purified DNA is released in the elution buffer. The sbeadex blood kit is supplied with ready-to-use buffers. The processing time on the KingFisher 96 is approximately 30 minutes. Typically DNA yields in the region of 5 µg are achieved with the sbeadex blood/ KingFisher 96 protocol from 200 µL of EDTA blood.

The method described here is a blood protocol which can be used for blood samples containing standard anticoagulants (citrate, EDTA, heparin) without adaptation. Whenever necessary, customisation of the protocol is possible using the software provided with the instrument.

Notes

- A copy of the instrument protocol is available on request (email: extraction@lgcgenomics.com)
- The instrument protocol is compatible with the KingFisher software version 2.6.22
- For tips and advice on how to adapt the instrument protocol for the BindIt™ software of the KingFisher Flex instrument please email extraction@lgcgenomics.com
- See the sbeadex blood kit protocol for further information about the kit, limitations of product use, safety information etc.

Equipment and reagents

Product description	Cat. No.	Labware required per run
sbeadex® blood kit (96 tests)	41403	-
sbeadex® blood kit (960 tests)	41430	-
KingFisher 96 magnetic particle processor	5400500*	-
KingFisher 96 DW magnet	24073430*	-
KingFisher 96 tip comb for DW magnet	97002534*	1
KingFisher 96 KF plate 200 µL	97002540*	1
DW 96 plate, V-bottom, polypropylene	95040450*	4
Ultrapure water (not part of the kit)	User supplied	-
1-Propanol (not part of the kit)	User supplied	-

Table 1: Equipment and reagents required for DNA extraction using sbeadex® blood kits on KingFisher 96.

* supplied by Thermo Fisher Scientific

Importing instrument protocol

To save the instrument protocol to your computer:

1. Open KingFisher software
2. Select **Cancel** in the Startup window
3. Select **Protocol** → **Import/Export data**
4. Click **Read file** on the left side of the 'Import/Export protocols' window. An 'Open' window appears
5. Select the protocol you want to import ('**sbx_blood_KF96.kf2**') and click **Open**
6. The protocol appears in the 'Protocols in file' list
7. Select protocol '**sbx_blood_KF96.kf2**' in the 'Protocols in file' list and click **Import**
8. A message will appear that the update of the database was successful
9. Now you can start the protocol directly from the software or transfer it to the KingFisher 96 instrument
10. Select **Instrument** → **Send protocol to Instrument**
11. Select the protocol ('**sbx_blood_KF96.kf2**') from the list 'Protocols for selected instrument' and click **Send protocol**
12. After the transfer of the protocol to the KingFisher 96 instrument a message will appear indicating the successful transfer.

Instrument procedure sbeadex blood kit

1. Fill the following deep well/ KingFisher plates with sbeadex® blood kit reagents as specified in table 2:
 - Add 200 µL of the blood samples into wells of a deep well plate 'Binding_Pos1'
 - Plate 'Wash1_Pos2'
 - Plate 'Wash2_Pos3'
 - Plate 'Wash3_Pos4'
 - Plate 'Elution_Pos5'
2. Add 170 µL of Lysis buffer BNM, 30 µL of Lysis additive and 20 µL of Protease solution to each blood sample and mix thoroughly. Alternatively you can prepare a premix of Lysis buffer BNM, Lysis additive and Protease for the corresponding sample number and add 220 µL of the premix to each sample. (Note: Use the premix immediately after preparation to prevent any decrease / loss in protease activity)
3. Cover the plate and incubate it at 55 °C for 10 min
4. Cool down to room temperature and add 250µL of 1-Propanol and 60 µL of sbeadex® particle suspension BN to each sample. Ensure the magnetic particles are re-suspended thoroughly before dispensing
5. Select the **sbx_blood_KF96** protocol on the KingFisher instrument
6. Load the prepared plates as prompted by the software and start the instrument
7. After approximately 30 min the protocol will be finished and the genomic DNA is ready for downstream analysis.

Plate name in protocol	Plate type	Well content	Volume
Comb_Pos6	KingFisher 96 KF plate	Tip comb	-
Binding_Pos1	DW 96 plate, V-bottom	Lysate	420 µL
		1-Propanol	250 µL
		Particle suspension BN	60 µL
Wash1_Pos2	DW 96 plate, V-bottom	Wash buffer BN 1	600 µL
Wash2_Pos3	DW 96 plate, V-bottom	Wash buffer BN 2	600 µL
Wash3_Pos4	DW 96 plate, V-bottom	Ultrapure water	600 µL
Elution_Pos5	DW 96 plate, V-bottom	Elution buffer BN	200 µL

Table 2: Plate filling instructions for KingFisher 96 and sbx_blood_KF96 protocol.



www.lgcgenomics.com

LGC Genomics

Germany
Ostendstr. 25 • TGS Haus 8
12459 Berlin

Tel: +49 (0)30 5304 2200
Fax: +49 (0)30 5304 2201
Email: info.de@lgcgenomics.com

United Kingdom

Unit 1-2 Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ

Tel: +44 (0) 1992 470757
Fax +44 (0) 1438 900670
Email: info.uk@lgcgenomics.com

USA

100 Cummings Center • Suite 420H
Beverly • MA 01915

Tel: +1 (978) 232 9430
Fax: +1 (978) 232 9435
Email: info.us@lgcgenomics.com