Manual

sbeadex blood kit
ThermoFisher KingFisher Flex 96

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

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

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Contents

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1. Introduction

sbeadex blood kits from LGC, Biosearch Technologies™, use magnetic separation for the purification of nucleic acids from 200 µL blood samples, including EDTA, heparin and citrate anticoagulants and buffy coat preparations. Superparamagnetic particles coated with sbeadex surface chemistry are used to capture nucleic acids from the blood sample, and utilise a novel two step binding mechanism which, when combined with the washing steps, removes impurities present in the sample matrix. After washing the nucleic acid is eluted and is ready for use in downstream processes. This kit is intended for research use only. It is not intended for use in diagnostic procedures.

The following protocol has been optimised for use with the ThermoFisher™ Kingfisher™ Flex 96. It requires only minimal hands on time. The addition of the blood sample, lysis components and binding/ sbeadex bead mixture are performed manually with all other tasks completed on board the instrument. For details on running a non-automated protocol, please see Section 4.

2. Kit contents and storage conditions

Please see Table 1 for the scales of the sbeadex blood kits which can be ordered:

Cat no.	Geographical region	Number of purifications per kit*
NAP44401	RoW (excl. APAC)	96
NAP44404	APAC	96
NAP44410	RoW (excl. APAC)	960
NAP44440	APAC	960
NAP44100	RoW (excl. APAC)	5,000
NAP44400	APAC	5,000

Table 1. Catalogue numbers for sbeadex blood kits. RoW = Rest of the world; APAC = Asia-Pacific. For research use only. Not for use in diagnostic procedures.

*The number of purifications is based on the Biosearch Technologies recommended protocol which assumes 200 µL starting blood volume.

All kit components should be used by the expiry date stated on the kit box, and stored under the recommended storage conditions. Please see Table 2 for details.

Component	Colour	Storage conditions
Lysis buffer SB	Blue	Room temperature
Protease solution	Grey	4 ºC
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)

Table 2. sbeadex blood kit components and storage conditions.

3. Experimental procedure

3.1 General information before starting

The protocols shown here are for the extraction of 100 µL or 200 µL of whole blood. If extracting buffy coat samples, prior optimisation of the protocol may be necessary. Please contact our Technical Support Team (see Section 7) for further guidance.

All processes are to be carried out at room temperature (15 °C-25 °C), unless otherwise stated.

To increase speed and efficiency of the protocol, and to minimise the drying-out of reagents (especially when working with smaller volumes) it is recommended to use a multichannel pipette, where appropriate.

It is important to ensure that the sbeadex particles suspension are properly resuspended before adding to the Binding buffer SB. Using a non-homogenous sbeadex beads suspension will affect the efficiency of the purification chemistry, potentially resulting in lower yields.

A timeline of the protocol run-time for the sbeadex blood kit purification is shown in Table 3:

Step	Process	Temperature	Incubation time on KingFisher Flex 96
1	Manual initial preparation steps (see Section 3.3)		
2	Manual lysis step preparation		
3	Loading of pre-loaded plates on instrument		
4	Lysis	70 °C	20 minutes
5	Manual adding of binding mix (see Section 3.3)		
6	Binding	Room temperature	4 minutes
7	Wash 1	Room temperature	5 minutes
8	Wash 2	Room temperature	5 minutes
9	Wash 3	Room temperature	5 minutes
10	Elution	70 °C	10 minutes

Table 3. Protocol run times for the sbeadex blood kit purification. The times stated are the actual incubation times for each stage, and do not take into account any transition times by the instrument. The total run time is approximately 59 minutes.

For information on carrying out the sbeadex blood kit protocol manually, see Section 4 of this manual.

3.2 Required materials (not included)

a) 2x Kingfisher 96-well standard plate
b) 4x Kingfisher 96-deep well plate
c) 1x Kingfisher 96 tip comb
d) 1x Kingfisher 96-deep well magnetic head
e) 1x Kingfisher Flex 96 96-deep well heat block
f) Suitable low-evaporation, semi-permanent plate seals, for use if reagents are to be dispensed in to blocks ahead of time for multiple runs. It is not recommended to heat-seal the pre-loaded plates.
g) Plastic V-bottom troughs for reagent dispensing (optional)
h) RNase (optional – see Section 3.3)
i) *sbeadex_blood_100µL_flex.bdz* or s*beadex_blood_200µL_flex.bdz* BindIt protocol file (see Section 3.4)

3.3 Initial preparations

a) *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 until the precipitates have re-dissolved.

b) *Pre-run checks:* It is recommended to have the instrument switched on before turning on the computer and opening the BindIt software. If there are connection issues between the instrument and the computer, it is recommended to switch everything off, and attempt to switch on again. Once the computer is connected to the instrument:
   - Ensure the correct KingFisher 96-deep well heat block is loaded onto the instrument.
   - Ensure the KingFisher deck is clear of any previously run plates.

c) *Digestion with RNase (optional):* sbeadex beads co-isolate RNA during the purification, and RNA has been shown to influence downstream measurement chemistries and/or enzymatic processing. If RNA is shown to be affecting downstream processes, it is recommended to add 600 U RNase per 100 mL Wash Buffer TN1.

d) *Binding buffer SB with sbeadex particles suspension (binding mix):* Mix the sbeadex beads thoroughly prior to use, to fully resuspend the particles, before adding to the appropriate volume of Binding buffer SB, following the guidelines in Table 4. It is recommended to prepare +10% final volume than is desired, to account for pipetting differences. The binding mix can be made in advance and stored tightly covered until use (8 hours at room temperature, 24 hours at 2-8 °C). Ensure the binding mix is equilibrated to room temperature before use.

Reagent	100 µL blood	100 µL blood (96 purifications +10%)	200 µL blood	200 µL blood (96 purifications +10%)
Binding buffer SB	160 µL	16.9 mL	320 µL	33.8 mL
sbeadex particles suspension	20 µL	2.1 mL	40 µL	4.2 mL
Binding mix per sample	**180 µL**		**360 µL**	

Table 4. Volumes for preparation of binding mix (Binding buffer SB with sbeadex particles suspension).

e) *Pre-loading of KingFisher plates with reagents:* If desired, the wash and elution reagents can be pre-loaded into appropriate KingFisher plates. The pre-loading should be performed on the same day as the purification:
   - Label 3x Kingfisher 96-deep well plates with Wash buffer BN1, Wash buffer TN1 and Wash buffer TN2, and label 1x KingFisher 96-well standard plate as Elution buffer AMP.
   - Fill the corresponding wells in each of the 96-deep well plates from step 2 (which matches the intended sample processing locations) with appropriate reagent, following the guidelines in Table 5.

Reagent	KingFisher plate type required)	Volume of reagent for 100 µL blood	Volume of reagent for 200 µL blood
Wash buffer BN1	96-deep well plate	400 µL	800 µL
Wash buffer TN1*	96-deep well plate	400 µL	800 µL
Wash buffer TN2	96-deep well plate	400 µL	800 µL
Elution buffer AMP	96-well standard plate	50-200 µL	

Table 5. Volumes of reagents to be pre-loaded into appropriate KingFisher plates prior to sample lysis.

*At this stage, RNase can be added to Wash Buffer TN1 to aid with downstream applications. Please see Section 3.3 for further details. If adding RNase to Wash buffer TN1 it is not recommended to pre-load these plates for >30 minutes prior to performing the purification.

NOTE: The plates should be sealed using a semi-permanent plate seal prior to running the protocol, to avoid contamination/evaporation. These seals should then be removed before loading the plates onto the instrument.

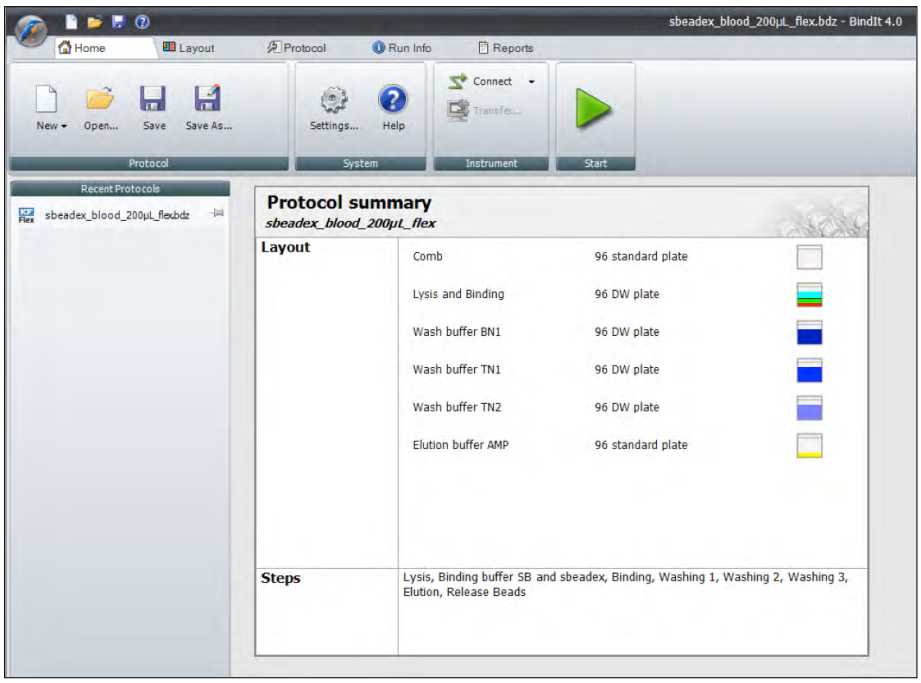
3.4 Automated sbeadex blood kit protocol (ThermoFisher KingFisher Flex 96)

In order to run the sbeadex kit protocol on the ThermoFisher KingFisher Flex 96, the *sbeadex_blood_100µL_flex.bdz* or *sbeadex_blood_200µL_flex.bdz* BindIt protocol is required. Please contact our Technical Support Team (details in Section 7), who will forward the correct BindIt (.bdz) file for your desired starting blood volumes.

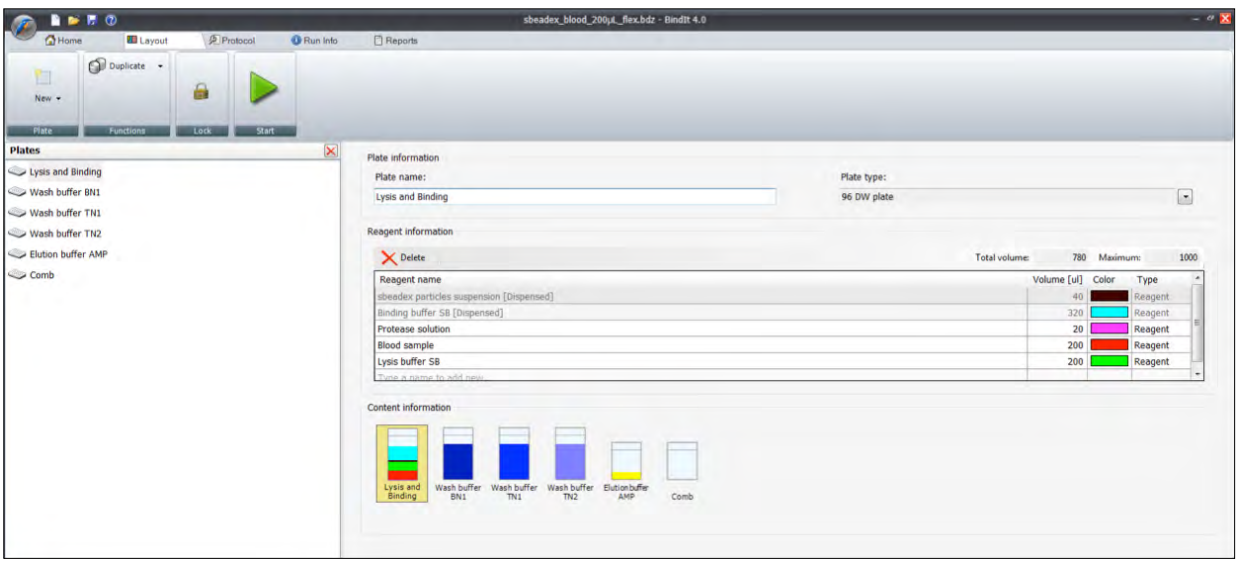
NOTE: The following screenshots are applicable for the sbeadex_blood_200µL_flex.bdz protocol. When using the sbeadex_blood_100µL_flex.bdz protocol, the layout and instrument progression are identical to the 200 µL protocol, other than the volumes of the reagents. The correct volumes will be pre-set in both BindIt (.bdz) files. Ensure the correct volumes of samples and reagents are loaded as per the starting blood volumes.

1. Use the BindIt software to open the appropriate *sbeadex_blood_[100µL or 200µL]_flex.bdz* protocol for your starting blood volume.

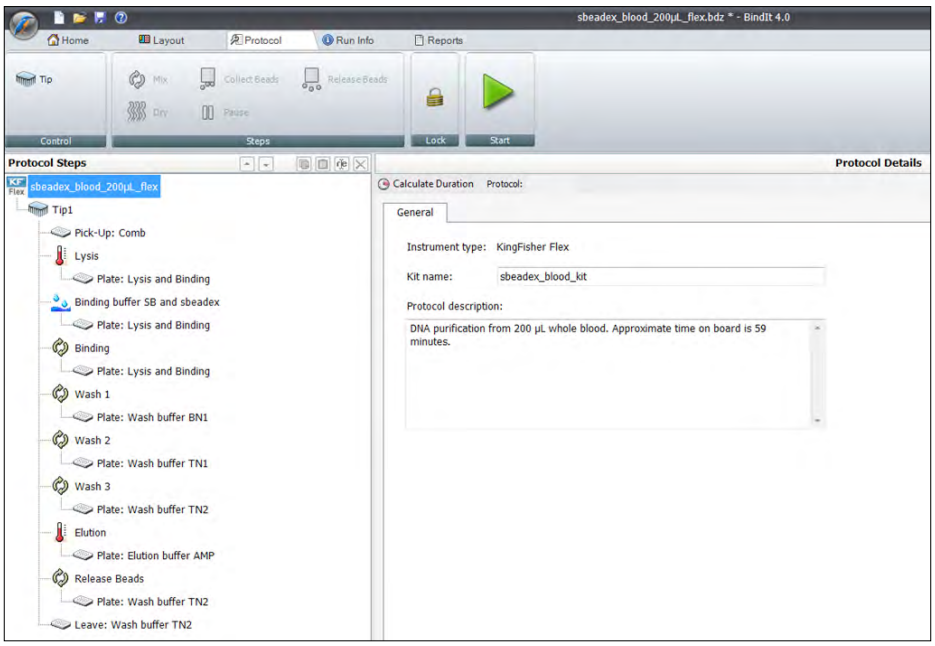
The “Home” screen will show a summary of the protocol, and the steps involved in the purification process.

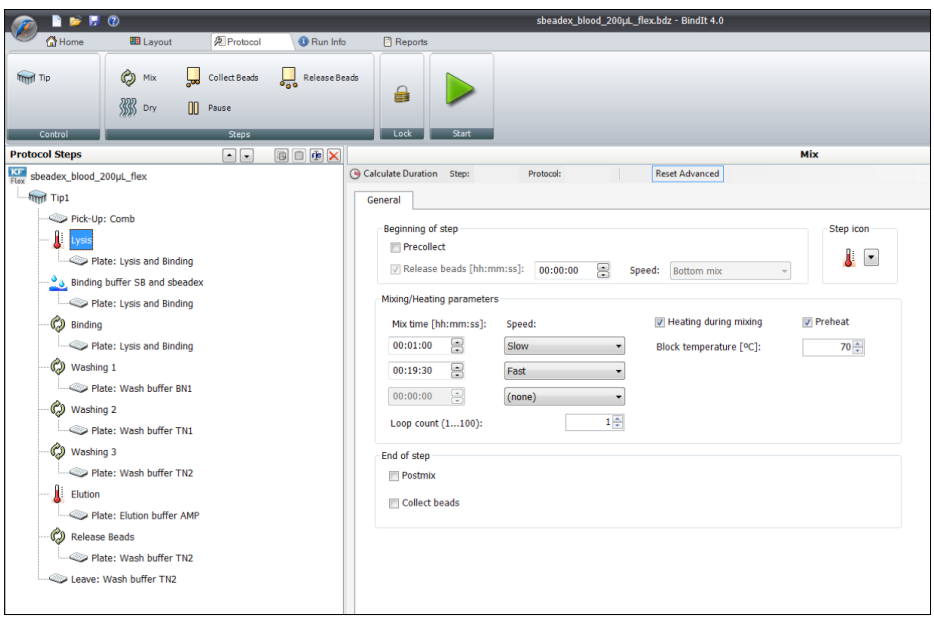


The “Layout” screen will show a summary of each of the plates involved in the purification process, and by selecting each of the “Plates”, the volumes of each reagent to be added to each plate will be shown. Please note that these volumes will differ between the 100 µL and 200 µL protocols.



The “Protocol” screen will show the steps in each process, and the details for each of the stages, including incubation times, incubation temperatures, mixing times and mixing speeds. Please see Table 3 for a summary of each of the stages in the purification protocol.





2. Ensure that 3x KingFisher 96-deep well plates and 1x KingFisher 96-well standard plate have been loaded with the appropriate volumes of reagents Wash buffer BN1, Wash buffer TN1, Wash buffer TN2 and Elution buffer AMP, respectively. Please see Section 3.3 for further details.
3. Load 1x KingFisher 96 tip comb into 1x KingFisher 96-well standard plate.
4. To a KingFisher 96-deep well plate, add the blood sample and lysis components, following the order of addition and volumes outlined in Table 6, dependent on the starting volume of the blood sample.

Order added to KingFisher 96-deep well plate	Reagent/sample	Volume of reagent for 100 µL blood	Volume of reagent for 200 µL blood
1	Protease solution*	10 µL	20 µL
2	Blood sample	100 µL blood	200 µL blood
3	Lysis buffer SB	100 µL	200 µL

Table 6. Order and volumes of lysis components and blood samples, dependent on the starting volume of blood sample.

* Care should be taken that small volumes of reagent/sample are added directly to the bottom of the reaction well to limit potential loss.

NOTE: After the addition of the Lysis buffer SB, the purification process should not be delayed for >5 minutes, as this may result in the Lysis buffer SB degrading the Protease solution. This may limit the efficiency of the lysis step, therefore affecting the final DNA yield recovered.

5. Press “START” on the KingFisher instrument.
6. The instrument will prompt to load the Kingfisher 96 tip comb on the instrument (from step 3). Load this plate on the instrument.
7. Load the pre-loaded reagents plates (Wash buffer BN1, Wash buffer TN1, Wash buffer TN2 and Elution buffer AMP from step 2) as prompted by the instrument, pressing “START” each time to load the next plate.
8. Load the prepared lysis plate on the instrument. Press “START”. The programme will run for approximately 20 minutes.
9. When required, the KingFisher software will then prompt for the addition of the binding mix (see Section 3.3).

NOTE: Ensure the binding mix is well mixed before adding to the appropriate wells on the lysis plate. If using a multichannel and a reagent trough to add the binding mix, it is recommended to periodically mix the binding mix with the pipette tips, to ensure the sbeadex beads remain homogenous in suspension.

10. Place the lysis plate (now with the binding mix added) back on the instrument. Press “START”.
11. The protocol will now run until completion.
12. Once the purification protocol is complete, remove the plates as instructed by the instrument. The plate labelled as Elution buffer AMP will contain the purified nucleic acid.

NOTE: It is advisable to seal the elution plate with an appropriate plate seal, to avoid loss of nucleic acid through evaporation.

4. Manual protocol for the sbeadex blood kit

This protocol can also be carried out manually. For a complete guide to running the sbeadex blood kit guide manually, please see the following link:

- sbeadex blood kit manual

5. Troubleshooting

If issues are being observed with the sbeadex blood 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, contact details in Section 7, who will be happy to assist you.

5.1 Common troubleshooting solutions

Problem	Possible cause	Possible solution
PCR inhibition	Inhibitors present in blood sample	Ensure only liquid blood is being used, minimising the clots when working with coagulated blood.
	Incomplete lysis	Contact our Technical Support team for further assistance.
Low yield	Inefficient binding	Ensure that the lysate, Binding Buffer SB and sbeadex particle suspension are mixed thoroughly.
	Excess of proteins limiting the DNA binding to the sbeadex beads	The protocol may benefit from an increased incubation time during the lysis step. Please contact our Technical Support Team for further assistance.
	Incomplete lysis	Contact our Technical Support Team for further assistance.
Coloured eluates	Incomplete lysis	Contact our Technical Support Team for further assistance.

Table 7. Common troubleshooting solutions.

5.2 Frequently asked questions (FAQs)

Frequently asked question	Possible solution
Can I use coagulated blood with the sbeadex blood kit?	Yes, however longer lysis times may be required in order to address possible inhibition. Contact our Technical Support team (see Section 7) for further guidance.
Is there a specific order of adding the Lysis buffer SB and the Protease solution to the sample?	Yes, it is recommended to add the components to the reaction tube in either, a) Protease solution then blood sample then Lysis buffer SB or, b) Lysis buffer SB then blood sample then Protease solution. Adding Protease solution directly to Lysis buffer SB may inactivate the activity of the Protease solution.

Table 8. Frequently asked questions (FAQs).

6. Safety information

- Wear appropriate skin and eye protection throughout the 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
- Safety data sheets are available for all kit components on request

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 9. Safety information for sbeadex blood kit components.

7. Further support

If you require any further support for the sbeadex blood kit and/or setting up the ThermoFisher KingFisher Flex 96 to run the sbeadex blood kit purification protocol, please do not hesitate to contact our Technical Support Team: biosearch.techsupport@lgcgroup.com

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