

# sbeadex livestock kit

Catalogue number **44701** and **44702**

(For research use only. Not for use in diagnostic procedures.)

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## 1. Introduction

The sbeadex™ livestock kit enables DNA extraction to be performed in parallel on a range of livestock sample types using one protocol. Sample types that can be extracted using this kit include blood, tissue (dried and preserved), hair, semen and saliva. Species that have been tested include:

- Bovine
- Chicken
- Dog
- Fish
- Goat
- Horse
- Sheep

Using this kit, high quality DNA can be extracted that is suitable for use in all downstream applications including PCR-based protocols and next generation sequencing.

sbeadex kits use superparamagnetic microparticles and a novel two-step binding mechanism to bind and purify nucleic acids. Combined with the washing steps, this unique process removes impurities and potential inhibitors of enzymatic reactions very effectively. The absence of any organic solvents in the final wash buffers prevents your nucleic acid preparation from being contaminated with inhibitory remains of these solvents and shortens the overall extraction time due to unnecessary drying and heating steps.



Figure 1. sbeadex protocols follow a unique technology which includes a two-step binding mechanism.

## 2. Kit contents and storage conditions

### 2.1 sbeadex livestock kit contents and storage conditions

The kit contents and storage conditions are outlined in Table 1 below (NAP44701 is sufficient for 96 extractions and NAP44702 is sufficient for 960 extractions). All kit components should be used within 12 months of receipt. Please refer to the kit box label for the expiry date.

Component	Label colour	Volume (NAP44701)	Volume (NAP44702)	Storage conditions
Lysis buffer SB	Blue	15 mL	200 mL	Room temperature
Lysis buffer PVP	Blue	15 mL	200 mL	Room temperature
Binding buffer SB	Green	30 mL	200 mL	Room temperature
sbeadex particle suspension + EDTA	White	2 mL	20 mL	Room temperature
Wash buffer BN1	Red	60 mL	500 mL	Room temperature
Wash buffer TN1	Red	60 mL	500 mL	Room temperature
Wash buffer TN2	Yellow	60 mL	500 mL	Room temperature (4 °C after opening)
Elution buffer AMP	Black	15 mL	100 mL	Room temperature (4 °C after opening)
Protease solution	Grey	1 mL	10 mL	Room temperature
Debris capture beads (DCB)*	Orange	4 mL	-	Room temperature (4 °C after opening)

Table 1. sbeadex livestock kit contents including volumes and required storage conditions.

\*Debris capture beads (DCB) are for use with particular sample types to assist with physical separation of debris after lysis. 40 µL DCB should be added per 1 mL Lysis buffer.

Additional buffers can be purchased separately if required (catalogue numbers available on request).

## 2.2 Formation of precipitates in buffers

Precipitates can form in the buffers at low temperatures. Always check for the presence of precipitates prior to use. If precipitates have formed, incubate the buffers at 37 °C for 10 minutes, and shake/stir thoroughly, to re-dissolve the precipitates.

## 2.3 Additional reagents that may be required

Depending on the sample type, some protocols may require additional reagents for optimal DNA extraction.

- **DTT**

This is typically required for sperm extraction. Without DTT, sperm is resistant to digestion due to the presence of protein disulphite bonds in their outer membrane. DTT should be added to Lysis buffer PVP (●) at a final concentration of 50 mM.

- *Digestion with RNase A*

sbeadex particles co-isolate RNA during the extraction procedure. The presence of RNA in extracted samples can interfere with certain downstream processes. In these instances, it is recommended to add RNase to Wash buffer TN2 (●) (600 U per 100 mL Wash buffer TN2). In cases where Proteinase is not used in the Lysis step, RNase should be added to Lysis buffer PVP (●) (600 U per 100 mL Lysis buffer PVP (●)).

- *Phosphate Buffer Solution*

If working with avian blood, PBS is required for dilution of blood prior to performing the lysis protocol. Avian blood should be diluted 1:40 in PBS.

## 3. Experimental procedure

### 3.1 Choose appropriate lysis protocol for sample type

The sbeadex livestock kit can be used to extract DNA from a wide range of sample types. Whilst the majority of the protocol steps are identical across sample types and can be run in parallel, it is recommended to follow the sample-specific initial lysis stage to ensure that optimal DNA extraction is performed.

Table 2 details the range of sample types that have been tested with this kit, and the recommended sample-specific lysis protocol. For any sample types not listed, please contact support for further information ([techsupport@lgcgroup.com](mailto:techsupport@lgcgroup.com)).

Sample type	Lysis protocol	Section in manual	Starting material
<b>Blood (Standard)</b>	Protocol A	Section 3.3.2.1	100 µL blood
<b>Blood (Protein rich)</b>	Protocol B	Section 3.3.2.2	50 µL blood
<b>Blood (Nucleated red blood cells)</b>	Protocol C	Section 3.3.2.3	50 µL blood
<b>Tissue (Dried) e.g. ear punches</b>	Protocol D	Section 3.3.2.4	Dried tissue Samples that have been stored in alcohol must be air dried before addition of lysis buffer. Samples stored in preservative must have the preservative solution removed
<b>Tissue (In preservative solution) e.g. Allflex samples</b>	Protocol E	Section 3.3.2.5	Tissue in preservative solution
<b>Hair root</b>	Protocol F	Section 3.3.2.6	10-60 hair roots
<b>Semen</b>	Protocol G	Section 3.3.2.7	10-50 µL semen (dependent on origin)
<b>FTA card/similar</b>	Protocol H	Section 3.3.2.8	3 x 6 mm punches
<b>Genotek swabs</b>	Protocol I	Section 3.3.2.9	Genotek swab – cells at bottom of tube
<b>Dry nasal/mouth swabs</b>	Protocol J/K	Section 3.3.2.10 Section 3.3.2.11	Dry swab
<b>Plant</b>	Protocol L	Section 3.3.2.12	20 mg wet tissue, lyophilised

Table 2. Sample-specific lysis protocols that should be used for each sample type.

### 3.2 Performing the protocol manually

When performing the sbeadex livestock kit DNA extraction protocol, a magnet or centrifuge is required to pellet the magnetic particles.

When removing supernatants, it is important to remove as much liquid as possible without dislodging the particle pellet. When placing the pipette tip inside the tube, ensure that the tip is aimed towards the front wall of the sample tube to avoid disruption of the particle pellet. It is recommended to aspirate once, let any liquid run down the walls of the tube, and then aspirate a second time to remove any remnants of liquid.

After the addition of each wash buffer, ensure the pellet is homogenised thoroughly in the wash buffer (e.g. by vortexing or pipetting up and down) to facilitate the best possible washing of the DNA.

If performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds to enable the magnetic particles to form a pellet.

For information on automation of the sbeadex livestock DNA extraction protocol, see Section 6 of this manual.

### 3.3. Laboratory procedure

#### 3.3.1. Before you start

##### 3.3.1.1 Reagent preparation

- *sbeadex particle suspension + EDTA*

Mix the sbeadex particle suspension + EDTA  thoroughly before use to fully re-suspend the particles.

- *Debris capture beads*

Mix the debris capture beads  thoroughly before use to fully re-suspend the particles

- *Protease solution*

Mix the Protease solution  thoroughly before use.

##### 3.3.1.2 sbeadex binding mix

Prior to performing the sbeadex livestock DNA extraction protocol, Binding buffer SB  can be combined with sbeadex particle suspension + EDTA  to create the binding mix. The table below details how to prepare a binding mix suspension for 1 and 100 extractions.

Component	Requirement for 1 DNA extraction	Requirement for 100 DNA extractions
Binding buffer SB 	160 µL	16 mL
sbeadex particle suspension + EDTA 	20 µL	2 mL

Table 3. Preparation of sbeadex particle suspension.

##### 3.3.1.3 Lysis buffer and Protease solution

If performing a large number of extractions, it may be beneficial to prepare a premix of Protease solution  and the relevant lysis buffer , sufficient for all extractions. This premix should be prepared immediately before use, and is not suitable for storage.

#### 3.3.2. Lysis protocol

This section contains the species-specific lysis protocols that should be performed prior to performing the extraction protocol in section 3.3.3. Refer to Table 2 (Section 3.1) to determine the appropriate lysis protocol for your sample type.

##### 3.3.2.1 Protocol A: Blood (Standard)

1. Add 100 µL Lysis buffer SB  and 10 µL Protease solution  to 100 µL blood and mix well.
2. Incubate at 55 °C for 20 min.
3. Cool samples to room temperature.
4. Proceed to extraction protocol (Section 3.3.3).

##### 3.3.2.2 Protocol B: Blood (Protein rich)

1. Add 50 µL Lysis buffer PVP  and 10 µL Protease solution  to 50 µL blood and mix well.
2. Incubate at 55 °C for 20 minutes.
3. Add 100 µL Lysis buffer SB  to 110 µL lysate. An additional 10 µL Protease solution  can be added at this stage if required. Mix well.

4. \*If additional Protease solution has been added: incubate at 55 °C for 20 min.  
\*No additional Protease solution has been added: optionally incubate at 60 °C for 5-10 min.
5. Proceed to extraction protocol (Section 3.3.3).

#### 3.3.2.3 Protocol C: Blood (Nucleated red blood cells)

1. Dilute blood 1:40 in PBS
2. Add 50 µL Lysis buffer PVP ● and 10 µL Protease solution ● to 50 µL diluted blood and mix well.
3. Incubate at 55 °C for 90 minutes.
4. Add 100 µL Lysis buffer SB ● to 110 µL lysate. An additional 10 µL Protease solution ● can be added at this stage if required. Mix well.
5. \*If additional Protease solution has been added: incubate at 55 °C for 90 min.  
\*No additional Protease solution has been added: optionally incubate at 60 °C for 5-10 min.
6. Proceed to extraction protocol (Section 3.3.3).

#### 3.3.2.4 Protocol D: Tissue (Dried)

Please note: Preservative must be removed. If stored in alcohol, tissue must be removed from solution and air dried before addition of lysis buffer.

1. Add an appropriate amount of Lysis buffer PVP ● and 10 µL Protease solution ● to tissue sample.  
Determination of the appropriate buffer volume will require some in-house testing - sufficient lysis buffer should be added to completely submerge the tissue sample and to ensure that the required volume of clear lysate can be easily removed after centrifugation (i.e. during Option 1, step f OR Option 2, step b).  
*\*Optional: 40 µL Debris capture beads (DCB) ● can be added to Lysis buffer PVP ● (40 µL DCB per mL Lysis buffer PVP).*
2. Incubate at 55 °C overnight or until tissue is dissolved. If feasible, shake the sample(s) occasionally during incubation.
3. Option 1:
  - a. Add 1:1 volume Lysis buffer SB ● (e.g. if 150 µL Lysis buffer PVP was used in step 1, add 150 µL Lysis buffer SB in this step)
  - b. Add 5 µL Protease solution ● and vortex.
  - c. Incubate at 55 °C for 1 hr.
  - d. Homogenise sample.
  - e. Spin at highest speed for 10 min.
  - f. Transfer 210 µL clear lysate to a new tube.
  - g. Proceed to extraction protocol (Section 3.3.3).

4. Option 2:

- a. Spin at highest speed for 10 min.
- b. Transfer 110  $\mu$ L clear lysate to a new tube.
- c. Add 100  $\mu$ L Lysis buffer SB ●.
- d. *Optional: Incubate at 60 °C for 5-10 min.*
- e. Proceed to extraction protocol (Section 3.3.3).

3.3.2.5 Protocol E: Tissue (Allflex, in preservative solution)

1. Add 1 volume\* Lysis buffer PVP\*\* ● and 10  $\mu$ L Protease solution ● to tissue sample in preservative solution.

*\*e.g. Add 100  $\mu$ L Lysis buffer PVP to tissue sample in 100  $\mu$ L preservative solution.*

*\*\*Optional: Debris capture beads (DCB) ● can be added to Lysis buffer PVP ● (40  $\mu$ L DCB per mL Lysis buffer PVP).*

2. Incubate at 55 °C overnight or until tissue is dissolved.

3. Option 1:

- a. Add 1 volume Lysis buffer SB ● and 5  $\mu$ L Protease solution ●.
- b. Incubate at 55 °C for 1 hr.
- c. Spin at highest speed for 10 min.
- d. Transfer 210  $\mu$ L clear lysate to a new tube.
- e. Proceed to extraction protocol (Section 3.3.3).

4. Option 2:

- a. Spin at highest speed for 10 min.
- b. Transfer 110  $\mu$ L lysate to a new tube.
- c. Add 100  $\mu$ L Lysis buffer SB ●.
- d. *Optional: Incubate at 60 °C for 5-10 min.*
- e. Proceed to extraction protocol (Section 3.3.3).

3.3.2.6 Protocol F: Hair root

1. Add 200  $\mu$ L Lysis buffer PVP ● and 10  $\mu$ L Protease solution ● to 10-60 hair roots.

2. Incubate at 55 °C for at least 1 hr (can incubate overnight).

3. Transfer 110  $\mu$ L lysate from the bottom of the well/tube to a new tube (N.B. small pieces of hair or debris from the hair roots can be included in the transfer without affecting the downstream protocol).

4. Add 100  $\mu$ L Lysis buffer SB to 110  $\mu$ L lysate.

5. *\*Optional: incubate at 60 °C for 5-10 min.*

6. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.7 Protocol G: Semen

1. Use 10-50  $\mu$ L semen (volume required dependent on semen origin).
2. Bring the total sample volume to 100  $\mu$ L with Lysis buffer PVP. Lysis buffer PVP ● should be supplemented with DTT at a final concentration of 50 mM.
3. Add 10  $\mu$ L Protease solution ●.
4. Incubate at 55 °C for at least 1 hr (can incubate overnight).
5. Option 1:
  - a. Add 100  $\mu$ L Lysis buffer SB ● and 5  $\mu$ L Protease solution.
  - b. Incubate at 55 °C for 1 hr.
  - c. *If semen has been diluted with an insoluble extender, and contains insoluble particles, spin at highest speed for 10 min, and proceed to step d.*  
*If semen has not been diluted with an insoluble extender, proceed directly to extraction protocol (Section 3.3.3).*
  - d. Transfer 210  $\mu$ L clear lysate to a new tube.
  - e. Proceed to extraction protocol (Section 3.3.3).
6. Option 2:
  - a. *If semen has been diluted with an insoluble extender, and contains insoluble particles, spin at highest speed for 10 min, and proceed to step b.*  
*If semen has not been diluted with an insoluble extender, proceed directly to step c.*
  - b. Transfer 110  $\mu$ L lysate to a new tube.
  - c. Add 100  $\mu$ L Lysis buffer SB ●.
  - d. *Optional: Incubate at 60 °C for 5-10 min.*
  - e. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.8 Protocol H: FTA card/similar

1. Prepare 3 x 6 mm punches
2. Add 130  $\mu$ L Lysis buffer PVP ● to punches.  
*Please note: If card contains semen samples, Lysis buffer PVP ● should be supplemented with DTT at a final concentration of 50 mM.*
3. Add 10  $\mu$ L Protease solution ●.
4. Incubate at 55 °C for 1 hr with constant shaking.
5. Add 130  $\mu$ L Lysis buffer SB ●.
6. Incubate at 55 °C for 1 hr with constant shaking.
7. Transfer 210  $\mu$ L lysate to a new tube.
8. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.9 Protocol I: Genotek swab

1. Using a cell saver tip (or tip with the end cut off), aspirate 110 µL solution from the Genotek tube to a new tube. Aspirate from the bottom of the Genotek tube to ensure gravity-pelleted cells are aspirated.
2. Add 100 µL Lysis buffer SB ●.
3. *\*Optional: Incubate at 60 °C for 5-10 min.*
4. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.10 Protocol J: Dry nasal/mouth swabs 1

1. Prepare a 1:1 solution of Lysis buffer PVP ● and Lysis buffer SB ●.
2. Add an appropriate volume of the solution prepared in Step 1 to the swab.  
A volume of 210 µL lysate is required for downstream steps of extraction, so if the swab absorbs ~200 µL buffer then a volume of >400 µL should be used.
3. Add 10 µL Protease solution ●.
4. Incubate at 55 °C for 1hr.
5. Spin at highest speed for 10 min.
6. Transfer 210 µL clear lysate to a new tube.
7. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.11 Protocol K: Dry nasal/mouth swabs 2

1. Add an appropriate volume of Lysis buffer PVP ● to the swab. A volume of 110 µL lysate is required for downstream steps of extraction, so if the swab absorbs ~200 µL buffer then a volume of >300 µL should be used.
2. Add 10 µL Protease solution ●.
3. Incubate at 55 °C for 1hr.
4. Spin at highest speed for 10 min.
5. Transfer 110 µL clear lysate to a new tube.
6. Add 100 µL Lysis buffer SB ● and mix well.
7. *\*Optional: Incubate at 60 °C for 5-10 min.*
8. Proceed to extraction protocol (Section 3.3.3).

### 3.3.2.12 Protocol L: Plant tissue

1. Grind lyophilised tissue in a tissue lyser (dry grind)
2. Add 200 µL Lysis buffer PVP\* ● to the dry ground tissue sample. For downstream extraction protocol, 110 µL lysate is required so volume of Lysis buffer PVP ● can be adjusted at this stage if required.  
*\*Optional: Debris capture beads (DCB) can be added to Lysis buffer PVP (40 µL DCB per mL Lysis buffer PVP).*

*\*\*Please note: for seed tissue, add 2 µL Protease solution ● per mL Lysis buffer PVP ●.*

3. Incubate at 55 °C for 1hr.
4. Spin at highest speed for 10 min.
5. Transfer 110 µL clear lysate to a new tube.
6. Add 100 µL Lysis buffer SB  and mix well.
7. *\*Optional: Incubate at 60 °C for 5-10 min.*
8. Proceed to extraction protocol (Section 3.3.3).

### 3.3.3. Extraction protocol

Before commencing the extraction protocol, ensure that samples have been lysed using the appropriate method as detailed in section 3.3.2. A volume of 210 µL lysate is required for the extraction protocol.

1. Add 180 µL binding mix   (Section 3.3.1.2) to 210 µL lysate  
Please note: If the expected quantity of DNA is very low, use 150 µL Binding buffer SB and 5 µL sbeadex particles + EDTA instead of standard binding mix.
2. Incubate at room temperature for 5 min with constant shaking (where possible).
3. Bring magnet into contact with the tube(s) for 2 min. sbeadex particles will form a pellet.
4. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible, and take care not to dislodge the pellet.
5. Add 400 µL Wash buffer BN1 . Mix thoroughly by pipetting (suggested pipette volume 350 µL) to fully resuspend the pellet.
6. Incubate at room temperature for 5-10 min. Periodically agitate the sample using a shaker or vortexer.
7. Bring magnet into contact with the tube(s) for 2 min. sbeadex particles will form a pellet.
8. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible, and take care not to dislodge the pellet.
9. Separate the magnet from the sample tube(s).
10. Repeat steps 5-9 with 400 µL Wash buffer TN1 .
11. Repeat steps 5-9 with 400 µL Wash buffer TN2 .

*\*Optional: If required, add RNase to Wash buffer TN2 as detailed in Section 2.3.*

### 3.3.4 Elution of DNA

1. Add 20-100 µL Elution buffer AMP  to the pellet. The volume of elution buffer required depends on the downstream DNA concentration requirements.
2. Mix thoroughly by pipetting (set pipette to half elution buffer volume) to fully resuspend the pellet.
3. Incubate at 55 °C for 10 min. Periodically agitate the sample using a shaker or vortexer.

Please note: Elution can be performed at room temperature if required. A 20 % reduction in DNA yield is typically observed when elution is performed at room temperature. An elution temperature of 55 °C is recommended to maximise DNA yield.

4. Bring magnet into contact with the tube(s) for 3 min.
5. Transfer the eluate to a new tube by pipetting. To avoid particle transfer it is recommended to transfer only 90 % of the eluate.

## 4. Troubleshooting

Table 4 below outlines common problems that can be experienced when performing DNA extraction using the sbeadex livestock kit. The possible causes are detailed, and corrective actions suggested.

Problem	Possible cause	Corrective action
<b>PCR inhibition</b>	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer. If necessary adjust the liquid handling parameters for automated systems.
<b>Low yield</b>	Inefficient binding	Ensure that the lysate, Binding buffer SB and sbeadex particle suspension are mixed thoroughly.
	DNA stays on beads (slimy pellet)	Too many proteins are attached to the DNA. Please follow the optional steps in the protocol. Too much DNA in sample. Re-elute with the same volume of Elution buffer.
	Insufficient lysis	Prolong digestion time, add reducing agent or in rare cases (e.g. hair samples) don't centrifuge samples after lysis as DNA sticking to partially digested debris might be spun down into the pellet.
<b>Coloured eluates</b>	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer. If necessary adjust the liquid handling parameters for automated systems.
	Heavily stained sample material	Incomplete lysis. Contact support ( <a href="mailto:techsupport@lgcgroup.com">techsupport@lgcgroup.com</a> ) for advice.
<b>Particles present in eluates</b>	Aspirating too fast	Reduce the speed ( $\mu$ L/sec) at which supernatants are removed.
	Loose pellet	Increase separation time (i.e. contact time with the magnet) to allow time for a tighter pellet to form.
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants.

Table 4. Troubleshooting common issues that may arise during the sbeadex extraction protocol.

## 5. Safety Information

- Wear appropriate skin and eye protection throughout the extraction procedure
- Lysis buffer SB , Binding buffer SB  and Wash buffer TN1  contain high concentrations of detergent and salt.  
Please note: In case of accidental contact, thoroughly rinse or flush the affected areas with water.
- Binding buffer SB  and Wash buffer TN1  contain up to 50 % n-propanol. Keep away from naked flames.
- Safety data sheets for all buffers included within the sbeadex livestock kit are accessible from LGC's website.

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
Lysis buffer PVP	●	! Warning	H319/H400 P101/P102/P103/P264/P273/P280/P305+P351+P338/P337+P313/P501
Binding buffer SB	●	! Danger	H226/H302/H315/H318/H336/H400 P101/P102/P103/P210/P241/P303+P361+P353/P305+P351+P338/P310/P501
Wash buffer BN1	●	! Danger	H226/H315/H318/H336 P101/P102/P103/P210/P303+P361+P353/P305+P351+P338/P310/P405/P501
sbeadex particle suspension + EDTA	○	-	-
Debris capture beads (DCB)	○	-	-
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	●	-	-
Protease solution	●	! Danger	H334/H317 P101/P102/P103/P261/P304+P341/P501

Table 5. Overview of safety information for all buffers included within the sbeadex livestock kit.

## 6. Automating the sbeadex extraction protocol

Once the sbeadex DNA extraction protocol has been optimised for your sample type manually, it is possible to automate the procedure to increase throughput. LGC recommends following the manual protocol that you have developed with respect to the volumes of buffers to use when automating the protocol. If you would like to discuss options for automation in your laboratory, please do not hesitate to contact our extraction specialists at LGC (see Section 7). We are able to offer pilot studies and customised protocols where required.

### 6.1 Mixing of samples

To mix samples efficiently using an automated liquid handling system, LGC recommends the following:

1. Set the mixing volume to between 50 and 80 % of the volume to be mixed (instrument dependent).
2. For each mixing step, aspirate and dispense between 5 and 10 times (dependent on efficiency of the liquid handler).
3. Ensure aspirate and dispense speeds are low for mixing steps involving Lysis buffers and Binding buffer SB ● to prevent frothing.
4. Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete resuspension.
5. Where appropriate a premix of sbeadex binding mix (3.3.1.2) with Lysis buffer SB ● is possible.

## 6.2 Automation via Kingfisher

For automation on a Kingfisher system (or similar) we recommend the following:

1. Keep all volumes the same as for manual extraction, except for the elution volume. Due to evaporation on the Kingfisher unit, 20 µL additional Elution buffer should be added to the elution plate (Step 3.3.4 of the manual protocol).
2. The incubation for each bind and wash step should be a minimum of 5 minutes long to account for diffusion-dependent wash effects. Elution should be carried out at 70 °C for 5-10 minutes.
3. Prior to mixing for the binding, washing and elution steps, use the 'Release Beads' function with a 'bottom mix' for 10 seconds. Automated mixing should then be performed using the 'Fast' setting.

A standard Kingfisher protocol can be obtained by contacting support ([techsupport@lgcgroup.com](mailto:techsupport@lgcgroup.com))

## 6.3 Automation via oKtopure

The oKtopure software is supplied with a standard sbeadex livestock template that can be used for automation of the sbeadex livestock protocol. When testing the protocol, it is important to observe for the following:

1. Blocked tips due to clumping beads
2. Effective resuspension of the pellet after addition of wash buffer.

If one of these issues is detected, please contact support for advice ([techsupport@lgcgroup.com](mailto:techsupport@lgcgroup.com)).

Please note: the oKtopure is not intended for use with blood.

## 7. Further support

If you require any further support with use of the sbeadex livestock kit, or would like to discuss options for optimisation, please do not hesitate to contact our team of nucleic acid extraction specialists.

**Telephone:** +49 (0)30 166354600

**Email:** [techsupport@lgcgroup.com](mailto:techsupport@lgcgroup.com)

 @LGCBiosearch | [biosearchtech.com](http://biosearchtech.com)



**Science for a safer world**