Manual

sbeadex Lightning Cell DNA Kit

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BIOSEARCH™ TECHNOLOGIES

Manual

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Contents

1. Purpose of this document

The purpose of this document is to provide a general introduction to the sbeadex™ Lightning Cell DNA Kit and details about how to use this kit in your laboratory. The document also contains a specific laboratory protocol required to manually perform nucleic acid purification from human or animal cells as well as information and guidance on the automation of the protocol. The sbeadex Lightning Cell DNA Kit is optimised for DNA isolation from up to 1 × 10^6 human or animal cells per reaction, with options for upscaling and downscaling the protocol outlined in sections 4.10 and 4.11.

2. Introduction to sbeadex Lightning chemistry

sbeadex Lightning uses superparamagnetic microparticles and a novel binding mechanism that allows for simultaneous binding and washing of DNA. Combined with a single water washing step, this unique process removes unpleasant wash steps with hazardous ethanolic or high chaotropic salt buffers. Impurities and potential inhibitors are efficiently removed leading to pure and high-quality DNA. sbeadex Lightning supplies clean, automatable purifications at the speed of crude extraction methods.

The sbeadex Lightning chemistry delivers nucleic acids of high yield, purity and quality that are suited for many downstream applications including PCR, qPCR, sequencing, NGS and restriction analysis.

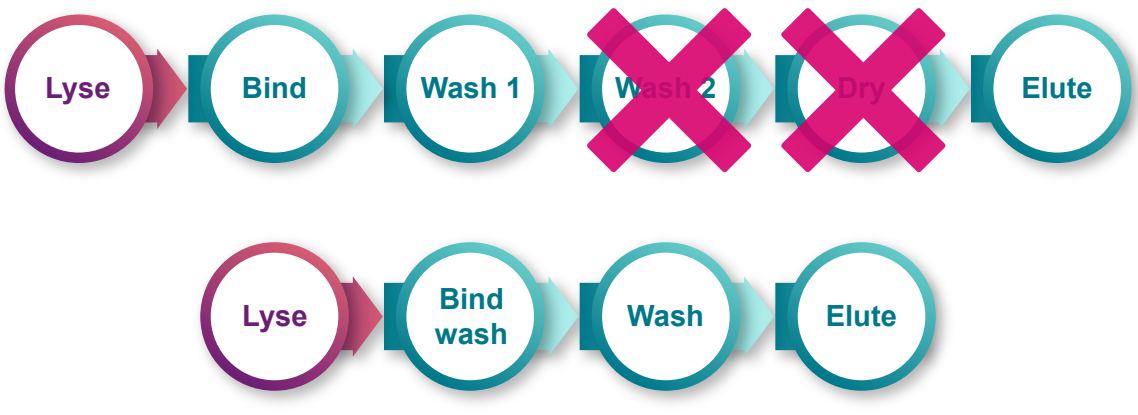

Figure 1. The shortened sbeadex Lightning workflow.
The upper workflow represents a typical magnetic bead-based DNA purification protocol.
The lower workflow illustrates the innovative sbeadex Lightning protocol.

3. Kit contents and storage conditions

Component	NAP40-033-00 (10 preparations*)	NAP40-033-01 (96 preparations)	NAP40-033-1 (960 preparations)	Storage conditions
Lysis buffer CLA	1 mL	15 mL	110 mL	Room temperature
Protease K solution	10 µL	110 µL	1.1 mL	Room temperature
RNase A solution	55 µL	550 µL	5.5 mL	Room temperature**
Binding buffer LCL	2 mL	22 mL	220 mL	Room temperature**
sbeadex particle suspension	220 µL	2,2 mL	22 mL	Room temperature
Elution buffer AMP	2 mL	22 mL	220 mL	Room temperature

Table 1. Components supplied in the sbeadex Lightning Cell DNA Kit, including details of component volumes by product code.
* This kit (10 preparations) is for testing purposes only and is not available for purchase via our web shop.
** If the buffer precipitates during shipment, please follow the instructions in section 4.6.

4. Experimental procedure

4.1 General information before starting

The protocol can be carried out manually or on various automated DNA extraction platforms. Section 5 provides recommendations and guidance for using the manual protocol and section 6 covers details for using the automated protocol.

This section contains general information on the materials required for both manual and automated procedures, along with guidance on critical steps such as cell lysis and DNA elution. It includes recommendations to support protocol optimisation, including considerations for scaling cell numbers and reaction volumes to achieve optimal results.

Depending on the cell type and for higher cell counts (e.g. >2 × 10^5 cells), lysates may become highly viscous and challenging to handle with automated liquid handling platforms. This issue may be resolved by adding reducing agents such as TCEP. For further guidance, please refer to the troubleshooting section.

4.2 Required materials (not included)

The list below details the equipment and reagents that are required to perform the sbeadex Lightning Cell DNA Kit protocol in your laboratory.

Essential

- Reaction tubes (1.5 mL or 2 mL) or plate(s) for manual protocol (see section 5)
- Plate(s) for the respective automation platforms (see section 6)
  - If working with the oKtopure™ we recommend Cat# KBS-7001-130 plates
  - If working with the KingFisher™ we recommend KingFisher Deep-well DNA purification plates, KingFisher Microplates (200 µL) and combs.
- Magnetic rack (for tubes or plates)
- Vortex mixer
- Centrifuge
- Equipment for heated incubation of samples during lysis and elution steps. This could be a water bath or incubator capable of temperatures up to 60 °C. A thermoshaker is preferred for efficient elution in tubes.
- 1X PBS
- Desalted or ultrapure water (pH below 7, nuclease-free)

Optional

- TCEP may help reduce viscosity in lysates with high DNA content and facilitate automation (see section 4.9.2 and the troubleshooting section).

4.3 Magnets and alternatives

When performing the sbeadex Lightning cell DNA kit protocol, a magnet or centrifuge is required to pellet the magnetic particles. Whilst use of magnets is recommended, if you are performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds at the highest possible speed to enable the magnetic particles to form a pellet.

4.4 Particle resuspension

It is important to ensure that the sbeadex particle suspension is properly re-suspended before adding it to the Binding buffer LCL for preparing a DNA binding premix or directly to the samples (see section 5). Using a non-homogenous sbeadex particle suspension will affect the efficiency of the purification chemistry, potentially resulting in lower nucleic acid yields and less uniform results.

4.5 Formation of precipitates in lysis buffer

Salt precipitates can form in the lysis buffer at low temperatures. Always check for the presence of precipitates prior to use. If precipitates have formed, incubate the buffer at 55 °C for 30 minutes, and shake thoroughly to re-dissolve the precipitates.

4.6 Coloration of binding buffer and precipitates

In Binding buffer LCL, coloration can occur over time. This is normal and does not impact performance. The binding buffer can also precipitate if stored too warm. This can be reversed by shaking and cooling the solution to 8 °C. Allow the binding buffer to reach room temperature before use. Low amounts of precipitates will not affect performance. Moderate shaking of the binding buffer before usage is recommended.

4.7 Laboratory conditions

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

4.8 Starting material and storage

sbeadex Lightning Cell DNA Kit enables DNA purification over a range of cell numbers and cell types of human or animal cells. The sbeadex Lightning Cell DNA Kit is optimised for DNA isolation from up to 1 × 10^6 human or animal cells per reaction, with options for upscaling and downscaling the protocol outlined in sections 4.10 and 4.11. Either fresh or frozen cells can be applied to the lysis reaction.

If storing cell pellets prior to DNA purification, we recommend removal of as much cell culture media as possible followed by immediate freezing of cell pellets to achieve optimal results. Frozen cell pellets should be stored at -80 ºC.

For information regarding lysis of adherent cells in culture dishes, please refer to section 4.9.2.

4.9 Sample collection and lysis

Both fresh and frozen cells should be resuspended in 1X PBS (100 µL for standard protocol) prior to performing the lysis reaction.

4.9.1 General recommendations for lysis optimisation

The sbeadex Lightning cell DNA kit is suitable for up to 1 × 10^6 human or animal cells per lysis reaction, with options for upscaling the protocol outlined in section 4.11. Using higher cell counts may result in viscous lysates. TCEP addition may help reduce viscosity in certain cell types.

Please note: the 1 × 10^6 cell number per lysis reaction relates to the manual protocol and may need to be reduced when using an automated protocol (see section 4.9.2 and section 6).

The standard lysis incubation time is 5 minutes at 60 °C. Lysis time may require adjustment for different cell counts and cell types. Prolonged lysis e.g. 10 minutes at 60 °C could be beneficial for efficient cell disruption in some cases.

High RNA concentrations in the eluate could lead to overestimation of DNA concentration when using UV-Vis based DNA concentration measurement methods and might interfere with downstream analyses. Depending on cell type, consider a prolonged cell lysis reaction e.g. 10 minutes for efficient RNase digestion.

4.9.2 Lysis procedure for plate-based manual and automated DNA purification protocols

This section provides guidance for collection of cells from multi-well culture plates to perform the lysis reaction in a DNA purification working plate using a starting volume of 100 µL cell suspension. Generally, both manual and automated DNA purification can be performed in a plate format if a suitable magnetic plate is available to enable sbeadex particles to form a pellet.

Suspension cells

To ensure optimal DNA purification, it is important to remove all cell culture medium prior to lysis. Cell culture media can interfere with DNA purification, so it is recommended to pellet the cells and carefully remove the entire supernatant.

Resuspend the cell pellet in 1X PBS, then transfer the resuspended cells into the DNA purification working plate and proceed immediately with the lysis reaction.

Adherent cells

(A) Lysis of detached cells after treatment with trypsin or similar detachment reagent

To detach adherent cells for subsequent DNA purification, apply an appropriate volume of trypsin or a comparable detachment reagent to the cell culture vessel. Following incubation and confirmed cell detachment, add 1X PBS to achieve a final volume of 100 µL per well (applicable to 24-well plates and smaller format plates). Resuspend the cells thoroughly within the original culture well and transfer the entire suspension to the DNA purification working plate. Proceed immediately with the lysis and DNA purification protocol.

Note: Components present in fully supplemented cell culture media (e.g., fetal calf serum) may interfere with efficient cell lysis and DNA binding and should therefore be avoided at this stage.

(B) Direct lysis of adherent cells on cell culture dish with subsequent DNA purification

Direct lysis of adherent cells in small-scale plate formats (24-well plate, 48-well plate or 96-well plate) is possible and trypsinisation could be omitted to expedite the protocol and increase throughput.

Remove culture media completely and, ideally, wash cells with 1X PBS to remove residual cell culture media. For 24-, 48- and 96-well plate formats, we recommend using 100 µL of supplemented Lysis buffer CLA per well (supplemented with 1 µL Protease K Solution, optional: 5 µL RNase Solution per reaction). Incubate plate for 5-10 minutes at room temperature or at up to 60 °C.

After lysis, transfer cell lysate to a DNA purification working plate for subsequent DNA purification. To efficiently transfer cell lysates, it might be necessary to increase lysate volume with a buffered solution (e.g. 1X PBS) to avoid extreme viscosity. By adding 100 µL 1X PBS per well, wells will fill to a total volume of 200 µL. This 200 µL volume can then be transferred completely into the DNA purification working plate before proceeding to the DNA binding step. If viscosity and inefficient transfer of lysates is observed, please refer to the troubleshooting section and recommendations for addition of TCEP to the lysis reaction.

To adjust the cell lysate volume according to the format of the cell culture plate, maintain a 1:1 (v/v) ratio of lysate to binding buffer, as outlined in section 4.11. Please note that this ratio may require optimisation depending on the specific cell type and intended downstream application. For technical support or guidance with protocol adjustments, please contact our nucleic acid extraction specialists (refer to section 8 for contact details).

4.10 Optimising DNA yield and concentration from high and low cell numbers

Both high and low cell counts may need slight protocol adaptations to optimise DNA purification results.

(A) High cell counts (>1 × 10^6 cells) might lead to poor DNA recovery from sbeadex particles due to inefficient DNA elution.

For higher cell counts, please consider

a. increasing Elution buffer AMP volume (up to 200 µL per reaction and elution step)
b. applying a second elution step with another 100-200 µL Elution buffer AMP to achieve highest possible final DNA yield
c. prolonging the elution time at 60 °C and using constant shaking (e.g. 1000 rpm) for more efficient DNA elution
d. applying shearing forces. i) lysate could be sheared after lysis, using a 1 mL pipette tip to pipette up and down, before proceeding with DNA purification as per section 5; ii) performing extensive vortexing of DNA eluate, including sbeadex particles, after the initial elution process. Both these techniques, utilised singularly or in conjunction, may help to release DNA bound to the beads.

In addition to the options above, prolonged lysis (e.g. 10 minutes at 60 °C) may help to achieve better DNA yield and quality.

If extreme viscosity is observed, addition of TCEP may help to overcome this limitation. Please refer to the troubleshooting section.

(B) Lower cell counts (<3 × 10^3 cells) might lead to low concentrated DNA eluates.

For low cell counts, omitting the RNase digestion might lead to higher DNA yields as RNA could serve as a carrier. If this is not an option due to the required downstream application, other carriers such as polyA or tRNA could be used.

For lower cell counts, and if a higher DNA concentration is desired, Elution buffer AMP can be scaled down to 30 µL per reaction while keeping all other reaction volumes constant as described in manual protocol (section 5).

Please also refer to the troubleshooting section.

4.11 Scalability of reaction volumes

For higher cell counts (>1 × 10^6 cells/reaction) upscaling the reagents may be beneficial, dependent on cell type. Either a fold-change of all reagents as described in protocol below (section 5) can be applied or the sbeadex Lightning cell DNA kit components can be upscaled if:

a. Cell suspension volume to lysis buffer volume is kept as a 1:1 (v/v) ratio
b. Lysate volume to binding buffer volume is kept as a 1:1 (v/v) ratio

Enzyme volumes must be upscaled accordingly.

The kit components should not be downscaled below the minimum volume described in the protocol (section 5).

The elution buffer volume can be up- or downscaled, depending on the desired effect.

5. Manual DNA purification protocol

Before you start with the protocol, please ensure neither lysis buffer nor binding buffer contain any precipitates. To remove precipitates please follow instructions in section 4.5 and 4.6.

1. Resuspend up to 1 × 10^6 human or animal cells (fresh or frozen) in 100 µL 1X PBS
2. Add 100 µL Lysis Buffer CLA (supplemented with 1 µL Protease Solution (20 mg/mL)). Lysis buffer and supplements can be prepared as a premix.

   Optional: add 5 µL RNase Solution (20 mg/mL) per reaction. Please note prolonged exposure of RNase to protease may lead to loss of RNase activity. Add RNase immediately prior to lysis reaction.
3. Mix sample by pulse-vortex (at least 3-5 times) and incubate for 5 minutes at 60 °C in a water bath or thermoshaker (1000 rpm). For lysis optimisation recommendations please refer to sections 4.9 and 4.10. After lysis it is crucial to pulse-vortex the lysate 3-5 times.
4. Add DNA binding mix consisting of 200 µL Binding buffer LCL and 20 µL well-resuspended sbeadex particle suspension per sample. DNA binding mix can be prepared as a pre-mix. If adding components separately, 20 µL of sbeadex particle suspension should be added to the lysate first, followed by 200 µL Binding buffer LCL.
5. Vortex sample for 30 seconds and allow sample to rest for an additional 30 seconds at room temperature.
6. After incubation, place sample on magnet. Allow particles to form a pellet, ensure sbeadex particles are pelleted completely and discard clear supernatant. (Optional: apply short spin to pellet sbeadex particles before placing sample on the magnet).
7. Add 400 µL of nuclease-free water to each sample.
8. Repeat steps 5 and 6.
9. Add 100 µL of Elution buffer AMP, vortex sample for 30 seconds and incubate for 3 minutes at 60 °C in a water bath or thermoshaker (1000 rpm). Please note that Elution buffer AMP volume can be scaled between 30 and 200 µL per reaction to achieve a desirable DNA concentration.

   For elution optimisation recommendations please refer to section 4.10.

10. Vortex sample for another 30 seconds.
11. Place sample on magnet, allow particles to form a pellet and ensure sbeadex particles are pelleted completely (Optional: apply short spin to pellet sbeadex particles before placing sample on the magnet). Transfer DNA eluate into a fresh tube. If particles are being transferred into DNA eluates, centrifuge sample prior to DNA quantification measurements.

    Note: Depending on cell count and DNA yield, sbeadex particles may travel slowly towards the magnet. If samples contain high DNA concentrations, a slight carryover of beads is possible. To remove any bead carryover, centrifuge the samples for 1 minute at maximum speed to pellet the beads and then carefully transfer the cleared eluate.

6. Automation

Before establishing an automated protocol for the sbeadex Lightning cell DNA Kit we recommend performing a manual isolation to optimise the purification protocol for your sample type(s), based on the recommendations in section 4.

The following section encompasses recommendations for different automation platforms (oKtopure (LGC, Biosearch Technologies, section 6.1), KingFisher System (Thermofisher, section 6.2), and other platforms (section 6.3).

For automation, Biosearch Technologies recommends using the same buffer volumes as described in the manual protocol (section 5). However, as described in section 4.11, the protocol is freely scalable and can be adjusted to your specific plate format.
If you would like to discuss options for automation in your laboratory, please do not hesitate to contact our nucleic acid extraction specialists for technical support (section 8). We also offer pilot studies and customised protocols where required.

6.1 oKtopure

The oKtopure (Biosearch Technologies) is a fully automated nucleic acid isolation platform that combines high-throughput automation with our proprietary sbeadex purification chemistry for high-quality and high-yield DNA purification. Full details about this platform can be accessed on our website. To discuss the use of oKtopure in your laboratory, please contact your local sales representative or our technical support team (section 8).

Lysates with high cell numbers might become very viscous, which could cause processing issues on this liquid handler platform. To reduce viscosity, consider adding TCEP (please refer to troubleshooting section).

For users with an established oKtopure platform, we offer a standard protocol for DNA purification from cells (<1 × 10^5 cells/reaction) using the sbeadex Lightning cell DNA kit. The following section provides a step-by-step protocol starting from cell suspension or cell lysate in a 96-well plate (for cell collection and preparation guidelines please refer to section 4.9).

Prepare the instrument accordingly for the run by priming the pump, homing the head, prefilling the tip boxes and filling the reservoirs with nuclease-free water and Elution buffer AMP. Place fresh elution plates where necessary.

Prepare your cell suspension in 100 µL total volume (according to section 4.9) and transfer into a DNA purification working plate (recommended plate: Cat# KBS-7001-130).

Pre-warm Elution Buffer AMP in a reservoir for elution-prompt procedure, which is part of the oKtopure protocol (see below).

Starting from 100 µL cell suspensions (with up to 1 × 10^5 cells/well) to be processed on oKtopure platform:

1. Add 100 µL Lysis buffer CLA (supplemented with 1 µL Protease K solution and, optionally, 5 µL RNase solution per reaction, see section 4.9) to each well, seal the plate with adhesive seal cover and mix by pulse-vortex 3-5 times.
2. Incubate plates for 10 minutes at 60 °C in an incubator to complete the lysis reaction.

Starting from 200 µL lysate volume

3. Add 220 µL Binding mix (consisting of 200 µL Binding buffer LCL and 20 µL sbeadex particle suspension per reaction) to each well.
4. Place plates onto deck together with reservoirs, appropriate tip boxes and elution plates for DNA eluates.
5. Ensure correct number of plates is inserted into the respective field in the oKtopure software and start the run.
6. With application of our recommended protocol, the instrument will pause and ask you to load a reservoir of pre-warmed Elution buffer AMP (elution-prompt procedure).
7. After the run, check the eluates for residual sbeadex particles. If there are visible residual sbeadex particles, seal and centrifuge the plate prior to performing DNA concentration measurements.

6.2 KingFisher Flex

The KingFisher™ Flex Purification System (ThermoFisher Scientific) enables fast automated DNA extraction from 24- or 96-well plates.

For cell collection and sample preparation guidelines please refer to section 4.9.

Biosearch Technologies offers a KingFisher Flex protocol (for 96-well plates) that starts from the binding step. We recommend performing cell lysis offline the instrument in an incubator for 10 minutes at 60 °C prior to performing the automated protocol.

Please note that for higher cell counts (>1 × 10^5 cells/well in a 96-well plate format), DNA elution may become inefficient. In the case of higher cell counts, we recommend a second elution step or an additional hands-on vortex step after elution on the KingFisher instrument to fully recover all DNA from the sbeadex particles. Note that our KingFisher protocol would need to be adapted accordingly. Please contact our nucleic acid specialists for technical support (section 8).

For protocol options, lysis-on-instrument and further technical support, please refer to section 8.

Prepare your cell suspension (according to section 4.9) and transfer 100 µL into a KingFisher Deep-well DNA purification plate.

Starting from 100 µL cell suspensions (with up to 1 × 10^5 cells/well) to be processed on KingFisher platform in 96-well format:

1. Add 100 µL Lysis buffer CLA (supplemented with 1 µL protease K solution and 5 µL RNase solution per reaction, see sections 4.9 and section 5) to each well, mix by pulse-vortex 3-5 times and seal the plate with adhesive cover.
2. Incubate for 10 minutes at 60 °C in an incubator to complete the lysis reaction.
3. Prepare all necessary plates: please consider recommendations for efficient DNA elution (section 4.10) and that low Elution buffer volumes (<100 µL per sample) are subject to evaporation on the instrument. Consider covering your elution plate with adhesive seal until the plate is loaded onto the instrument.
    a. wash plate: 96-well deep-well plate, 400 µL nuclease-free water per reaction
    b. elution plate: 96-well standard plate, 50-200 µL Elution buffer AMP
4. After lysis incubation, pulse-vortex lysis plate 3-5 times and add binding mix consisting of 200 µL Binding buffer LCL and 20 µL sbeadex particle suspension per reaction to each sample.
5. Insert comb into the DNA purification plate; the instrument will pick up the comb from this plate.
6. Place all plates onto the instrument and start the run.
7. After the run, unload the instrument and check DNA eluates for residual sbeadex particles. If there are visible residual sbeadex particles, seal and centrifuge the plate or put onto a magnet prior to performing DNA concentration measurements.

6.3 Other automation platforms

In general, the sbeadex Lightning cell DNA kit is compatible with other liquid-handler DNA purification platforms (e.g. Hamilton, Opentrons®, Beckman Coulter®, Dynamic Devices, Analytic Jena® or Tecan®). When establishing protocols, please do not hesitate to contact our nucleic acid specialists (see section 9) for guidance and support.

7. Troubleshooting

Problem	Solution suggestion
Viscous lysates	Consider prolonged lysis reaction. Consider addition and titration of TCEP with starting concentration from 5 mM and upwards. Please note that TCEP addition might result in overall reduced DNA yield but reduce lysate handling issues caused by lysate viscosity. Consider reducing or titrating cell count input per reaction.
Particles travel slowly towards magnet in tube-based protocol.	sbeadex particles might travel slowly towards magnetic field depending on the sample type, DNA yield and magnet strength. A short spin before bringing samples onto magnet will help sbeadex particles to pellet more quickly.
Poor 260/280 or 260/230 ratio in UV-Vis based DNA quantification measurement	Make sure to remove all residual cell culture media and consider washing cells prior to resuspending cell pellets in 1X PBS for starting lysis reaction. Thoroughly remove supernatants after binding and wash step prior to DNA elution during DNA purification protocol. If the 260/280 ratio is low (below 1.7), a prolonged lysis time e.g. 10 minutes at 60 °C might improve overall DNA purity.
Low nucleic acid yield	Prolong the lysis time e.g. 10 minutes at 60 °C. If low cell counts (<1 × 10^4 cells/reaction) are used, consider reducing the elution buffer volume down to 30 µL Elution Buffer AMP per reaction, whilst keeping all other volumes constant, to concentrate the DNA. Please also refer to section 4.10. If higher cell counts are being applied (>1 × 10^6 cells/reaction) and poor DNA elution is observed consider: a) reducing starting material b) optimising DNA elution step (see section 4.10) c) up-scaling reaction volumes (see section 4.11), specifically the elution volume (e.g. up to 200 µL) and the elution duration (see section 5). Carriers, such as polyA, for efficient DNA binding from low cell numbers (<3-5 × 10^3 cells per reaction, depending on cell type) could be beneficial.
Poor downstream results	Residual cell culture media might interfere with downstream assays. Consider washing cell culture with 1X PBS prior to lysis reaction. Quantify DNA with fluorescence-based DNA quantification methods in addition to UV-Vis based methods to evaluate DNA yield and purity more fully. Residual total RNA might lead to overestimation of DNA yield and, consequently, underloading in downstream analyses.

8. Further support

If you require any further support for any of the sbeadex products, please contact our technical support team at techsupport@lgcgroup.com.

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