Manual

sbeadex Lightning Plant DNA Kit
sbeadex Lightning Plant HTP DNA Kit

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

BIOSEARCH™ TECHNOLOGIES

Manual

sbeadex Lightning Plant DNA Kit and sbeadex Lightning Plant HTP DNA Kit

Contents

Manual

sbeadex Lightning Plant DNA Kit and sbeadex Lightning Plant HTP DNA Kit

1. Purpose of this document

The sbeadex™ Lightning Plant DNA Kit is available in two formats:

1. **Standard version (sbeadex Lightning Plant DNA Kit):** Designed for manual or automated DNA extraction from up to 100 mg of fresh plant material. Suitable for use in tubes or 96-well plates.

2. **HTP (high-throughput) version (sbeadex Lightning Plant HTP DNA Kit):** A miniaturised format optimised for 384-well plate processing, using reduced reaction volumes for high-throughput applications.

This manual includes a comprehensive laboratory protocol for the manual purification of nucleic acids from plant material. In addition, it provides detailed guidance on how to automate the protocol using compatible liquid handling systems, ensuring flexibility for both low- and high-throughput laboratory environments.

This user manual provides general information and detailed protocols for using the sbeadex Lightning Plant DNA Kit and the sbeadex Lightning Plant HTP DNA Kit. We strongly recommend reading this manual thoroughly before first use to ensure optimal performance.

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 DNA purification 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, quantitative PCR, sequencing, NGS and restriction analysis.

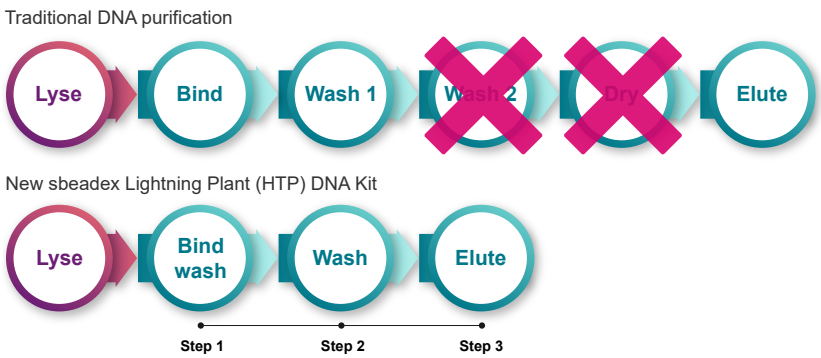

Figure 1. The shortened sbeadex Lightning workflow.
The upper workflow represents a typical magnetic bead-based DNA purification protocol. The lower workflow illustrates the innovative sbeadex Lightning protocol.

3. Kit contents and storage conditions

sbeadex Lightning Plant DNA Kit

Component	NAP40-035-00 (10 preps*)	NAP40-035-01 (96 preps)	NAP40-035-02 (960 preps)	NAP40-035-03 (10,000 preps)	Storage conditions
Lysis buffer PLA	4 mL	44 mL	440 mL	4,400 mL	Room temperature
Protease K solution (20 mg/mL)	10 µL	100 µL	1 mL	10 mL	Room temperature
Debris capture beads	180 µL	1.8 mL	18 mL	180 mL	Room temperature
Binding buffer LPL	2 mL	22 mL	220 mL	2,200 mL	Room temperature**
sbeadex particle suspension	220 µL	2.2 mL	22 mL	220 mL	Room temperature
Elution buffer AMP	1 mL	11 mL	110 mL	1,100 mL	Room temperature

Table 1. Components supplied in the sbeadex Lightning Plant 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 4.5.

sbeadex Lightning Plant HTP DNA Kit

Component	NAP40-036-01 (1536 preparations	NAP40-036-02 (10,000 preparations)	Storage conditions
Lysis buffer PLA	500 mL	2,200 mL	Room temperature
Protease K solution (20 mg/mL)	1 mL	4.4 mL	Room temperature
Debris capture beads	20 mL	88 mL	Room temperature
Binding buffer LPL	110 mL	600 mL	Room temperature
sbeadex particle suspension	11 mL	60 mL	Room temperature
Elution buffer AMP	100 mL	600 mL	Room temperature

Table 2. Components supplied in the sbeadex Lightning Plant HTP DNA Kit, including details of component volumes by product code.

4. Experimental procedure

Plant samples present a wide range of challenges due to their structural diversity, including tough cell walls, the presence of PCR-inhibitory secondary metabolites, and the sheer variety of tissue types. These factors make it difficult to address all sample types with a single standardised protocol. The following sections provide detailed guidance on individual protocol steps to help you adapt and fine-tune the procedure according to specific sample characteristics and customer needs.

4.1 Required materials (not included)

The list below details the equipment and reagents that are required to perform sbeadex Lightning technology based nucleic acid purification in your laboratory, in addition to the reagents supplied in the sbeadex Lightning plant (HTP) DNA Kit.

Essential

- Magnetic rack or centrifuge
- 96- or 384-well plates (deep-well format), or reaction tubes
- Centrifuge for plant samples and plates
- Water bath or incubator (capable of temperatures up to 60 °C)
- Desalted or ultrapure water (pH value below 7)

Optional

- RNAse A solution (20 mg/mL)

4.2 Magnets and alternatives

To pellet the magnetic particles during the protocol, a magnet, magnetic plate, or centrifuge is required. While the use of magnets is strongly recommended for optimal performance, manual processing without a magnet is also possible. In such cases, sample tubes or plates can be centrifuged at maximum speed for 10 seconds to ensure the magnetic particles form a compact pellet.

4.3 Particle resuspension

It is essential to ensure that the sbeadex particle suspension is thoroughly re-suspended before being added to Binding buffer LPL to prepare a DNA binding premix or when it is added directly to the samples. Using a non-homogeneous particle suspension can negatively impact the efficiency of the purification chemistry, potentially leading to reduced nucleic acid yields and inconsistent results.

4.4 Formation of precipitates in lysis buffer

Salt precipitates may form in the lysis buffer when stored at low temperatures. Always inspect the buffer for visible precipitates before use. If precipitates have formed, incubate the buffer at 55 °C for 30 minutes, and shake thoroughly to re-dissolve the precipitates.

4.5 Colouration of binding buffer and precipitates

In Binding buffer LPL, colouration can occur over time. This is normal and does not impact performance. The binding buffer can also precipitate if storage conditions are too warm. This can be reversed by shaking and cooling the solution to 8 °C. Low amounts of precipitates will not affect performance. Moderate shaking of the binding buffer before usage is recommended.

4.6 Laboratory conditions

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

4.7 Starting material and storage

The sbeadex Lightning Plant DNA Kit and sbeadex Lightning Plant HTP DNA Kit are optimised for a wide variety of plant sample types including leaf, tuber and seed. Plant samples can be processed fresh, frozen, dried, or freeze-dried. For long-term storage of plant samples, we recommend one of the following options:

Option A: Snap freeze and storage in a freezer
Plant samples can be effectively sampled and stored by snap-freezing them in liquid nitrogen and subsequently storing the frozen samples at -70 °C in a freezer.

Option B: Storage at room temperature
For storage at room temperature, it is recommended to thoroughly dry plant samples. In addition, samples should be protected from light.

To conveniently dry, store and ship plant and seed samples Biosearch Technologies offers BioArk Leaf and Seed Kits. These can be ordered separately from our web shop.

Option C: Lyophilisation
Plant samples can be lyophilised and subsequently stored for an extended time at room temperature.

4.8 Disruption and homogenisation of plant materials

Thorough homogenisation of the plant and seed material is crucial for an effective lysis reaction and release of DNA from the plant cells. Different homogenisation options are available depending on the throughput and storage conditions of the samples.

4.8.1 Manual grinding

Manual grinding using a pestle and mortar or a micropestle is a suitable method for processing small numbers of plant or seed samples. For fresh material, we recommend either adding lysis buffer during homogenisation or snap-freezing the samples in liquid nitrogen and grinding them while frozen. This approach helps to preserve DNA integrity and ensures effective cell disruption.

If high molecular weight (HMW) DNA is desired, gentle manual grinding or lyophilising the tissue prior to homogenisation is advised.

4.8.2 Ball mill grinding

For higher throughput, homogenisation using steel balls in tubes or plates with a ball mill is recommended. This method is efficient for processing large batches of samples. To optimise results, samples should be dried, freeze-dried or frozen before ball mill grinding, as this enhances disruption and DNA release.

Homogenisation efficiency is affected by various factors including sample type, amount of sample, type of homogenisation balls, and use of tubes or plates. As it has a major impact on the DNA yield and quality we recommend optimising the homogenisation process for each sample type.

4.9 Lysis recommendations and preparation of lysates for binding reaction

This kit includes a lysis buffer formulation that has been validated for effective performance across a wide range of plant leaf and seed sample types. The protocol outlines general guidelines that are typically successful for most sample types. However, for certain sample types, optimising the lysis conditions may be necessary to achieve the best results. If DNA yield or purity falls below expectations, fine-tuning the lysis parameters can be beneficial.

Several factors influence lysis efficiency:

4.9.1 Lysis time

A lysis duration of 10 to 60 minutes at 55 °C is recommended. In some cases, extending the lysis time beyond this range may improve results.

4.9.2 Lysis temperature

The optimal temperature for Protease K digestion is 55 °C. Note that the actual temperature within the sample may be lower than the set temperature on your heating device. If you are unsure whether your samples reach 55 °C during incubation, measure the temperature directly. If it is too low, increase the device settings to ensure optimal conditions.

4.9.3 Lysis buffer

The included Lysis buffer PLA is suitable for a broad spectrum of plant tissues and seed samples. However, some plant types may require alternative lysis buffers to efficiently release DNA and eliminate PCR inhibitors. LGC has developed a range of lysis buffers compatible with sbeadex Lightning chemistry. We offer a sbeadex Lightning Starter Kit (NAP40-032-00) containing six different lysis buffers, allowing you to tailor the lysis conditions to specific sample types.

Protease K concentration:
We recommend using 2 µL of Protease K solution (20 mg/mL) per 1 mL of lysis buffer. For certain samples, increasing the Protease K concentration may improve protein digestion efficiency.

For personalised guidance on optimising lysis conditions for specific sample types, please contact our technical support team (see section 10).

4.10 Optimising input amount and lysis buffer volume

The required sample amount and lysis buffer volume can vary depending on the type and condition of the plant material. Factors such as tissue type (leaf vs. seed), moisture content (fresh vs. dried), and sample preparation (see 4.8) can significantly influence lysis efficiency and lysate clarity. Therefore, some optimisation may be necessary to achieve consistent DNA yield and quality. When working with a new sample type, we recommend adjusting the lysis buffer volume accordingly to ensure optimal performance.

Recommended sample input per reaction:

sbeadex Lightning Plant DNA Kit

Up to 100 mg of fresh material or 20 mg of dried material can typically be used. These values may vary depending on the sample type and may require optimisation.

sbeadex Lightning Plant HTP DNA Kit

For this miniaturised high-throughput format we recommend not exceeding 30 mg of fresh material or 5 mg of dried material per reaction, depending on the sample characteristics.

When using fresh leaf samples, an input volume of 250-300 µL of Lysis buffer PLA typically produces 200 µL of clear lysate, which is required for the DNA binding step after lysis and centrifugation.

For dried seed or leaf material that may soak up a lot of buffer, a higher volume of lysis solution (e.g. 450-600 µL) may be required to obtain 200 µL of cleared lysate as input for the binding step. Please refer to protocol section 6.1 for preparation of the lysis solution.

Additionally, optimising the sample to lysis buffer ratio (e.g. using less than 400 µL of Lysis buffer PLA), as described in the protocol, may help concentrate nucleic acids and result in a higher final DNA concentration in the eluate.

4.11 Mixing

Proper mixing of magnetic beads and the sample is crucial during DNA binding, washing and elution steps.

4.11.1 Vortexing

If processing manually, vortexing is one of the most efficient ways to properly mix during the DNA binding, wash and elution steps. We highly recommend applying constant vortexing of samples for indicated timepoints instead of pulse vortexing.

4.11.2 Mixing on a shaker in a multi-well plate

All protocol steps can be performed in a multi-well plate format. The sbeadex Lightning Plant DNA Kit is compatible with 96-well plates, while the sbeadex Lightning Plant HTP DNA Kit is optimised for high-throughput processing in 384-well plates.

When using a shaker, we strongly recommend using deep-well plates to minimise the risk of cross-contamination during high-speed shaking. Before starting routine processing, it is advisable to establish and validate mixing parameters that ensure thorough and uniform mixing throughout the DNA binding, washing, and elution steps.

To test mixing efficiency and avoid cross-contamination, you can simulate the workflow using dyed water in a deep-well 96- or 384-well plate. This allows you to visually assess mixing performance and adjust speed or duration as needed.

Please note that mixtures containing sample lysate, Binding buffer LPL, and sbeadex particle suspension may exhibit higher viscosity and behave differently than water-based solutions. These mixtures may require modified mixing routines to achieve optimal results.

If you need assistance optimising your shaker-based mixing protocol, please contact our technical support team (see contact section 10).

4.11.3 Pipette mixing

Alternatively, mixing by pipetting up and down can ensure proper mixing during DNA binding, wash and elution. If you encounter processing limitations such as high viscosity or bead clumping, please consider using wide-bore tips for mixing the samples. Please also consider optimising your input sample amount and lysis buffer volume as outlined in section 4.10.

5. sbeadex Lightning short protocols

This section provides a condensed, one-page protocol intended for experienced users. If you are using the kit for the first time, we strongly recommend reading the full protocol in sections 6 and 7.

	sbeadex Lightning Plant DNA Kit tubes or 96-well plate format	sbeadex Lightning Plant HTP DNA Kit 384-well plate format
Homogenisation	Up to 100 mg fresh or 20 mg dry material	Up to 30 mg fresh or 5 mg dry material
Lysis	Lysis solution: 2 µL ProK[1] + 40 µL DCB[2] per 1 mL PLA Add 400 µL Lysis solution Incubate 30 minutes at 55 °C Clear lysate by centrifugation at max speed.	Lysis solution: 2 µL ProK[1] + 40 µL DCB[2] per 1 mL PLA Add 200 µL Lysis solution Incubate 30 minutes at 55 °C Clear lysate by centrifugation at max speed.
Binding	Combine: 200 µL cleared lysate 200 µL LPL 20 µL sbeadex particle suspension	Combine: 50 µL cleared lysate 50 µL LPL 5 µL sbeadex particle suspension
	Mix by pipetting, vortex or shaking	Mix by pipetting, vortex or shaking
	Magnetic separation Remove supernatant	Magnetic separation Remove supernatant
Wash	Remove from magnet Add 400 µL ultrapure water	Remove from magnet Add 100 µL ultrapure water
	Mix by pipetting, vortex or shaking	Mix by pipetting, vortex or shaking
	1 minute separation Remove supernatant	1 minute separation Remove supernatant
Elution	Remove from magnet Add 50-200 µL AMP	Remove from magnet Add 20-50 µL AMP
	Mix by pipetting, vortex or shaking Incubate for min 1 minute at 60 °C	Mix by pipetting, vortex or shaking Incubate for min 1 minute at 60 °C
	1 minute separation Transfer to fresh plate/tube	1 minute separation Transfer to fresh plate/tube

Table 3. sbeadex Lightning Plant and sbeadex Lightning Plant HTP short protocols.
[1] ProK: Protease K solution
[2] DCB: Debris capture beads

6. sbeadex Lightning Plant DNA Kit protocol for DNA purification

6.1 Sample homogenisation and lysis

- 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 4.4 and 4.5.
- Homogenise up to 100 mg fresh or up to 20 mg dried plant tissue (see section 4.8 for recommendations on homogenisation and section 4.10 for input optimisation).
- Prepare a lysis solution by adding 2 µL Protease K solution (20 mg/mL) and 40 µL debris capture beads per mL of Lysis buffer PLA.

  NOTE: A premix of lysis buffer, Protease K solution, and debris capture beads is stable for 24 hours at room temperature.

  NOTE: If your downstream application is sensitive to RNA carry-over a RNA digestion with RNase A may be beneficial to efficiently remove total RNA from your samples. We suggest using 5 µL of RNase solution per 1 mL of Lysis Buffer. Please note that RNase solution is not included in the kit. For ordering information please refer to section 4.1.

- Add 400 µL of the prepared lysis solution to the homogenised sample and mix thoroughly.

  NOTE: If fresh tissue has been used and Lysis buffer PLA was used during the homogenisation, no further addition of Lysis buffer PLA is required. Make sure to add 2 µL of Protease K solution (20 mg/mL) and 40 µL of debris capture beads per mL of Lysis buffer PLA before proceeding to the next step.

  NOTE: Depending on the amount of sample and the type, lysis buffer volume may be adapted. Ensure that you can transfer 200 µL of cleared lysate without disturbing the pellet after the lysis. Please see section 4.10 for recommendations on lysis buffer volumes.

- Incubate the sample at 55 °C for 30 minutes.

  NOTE: Depending on sample type, lysis may be shortened or prolonged (please refer to section 4.9 for more details).

  NOTE: Mixing or periodic mixing can be beneficial for an efficient lysis.

- After the incubation, centrifuge sample at maximum speed for 5 minutes to pellet the debris.

6.2 Bind

- Add 200 µL of Binding buffer LPL and 20 µL of sbeadex particle suspension to a fresh tube or well.

  NOTE: Ensure the sbeadex particle suspension is well resuspended before use.

  NOTE: A pre-mix of Binding buffer LPL and sbeadex particle suspension is stable for at least one month at room temperature.

- Transfer 200 µL of the cleared lysate to the binding mix.
- Vortex for 30 seconds and allow to rest for 30 seconds at room temperature.

  NOTE: Thorough mixing is essential for optimal performance. For manual processing, we recommend vortexing, though pipette or shaker-based mixing can also be used. Please refer to section 4.11 for guidance on suitable mixing methods.

- Bring the magnet into contact with the tube or plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Remove the supernatant and discard. Remove as much supernatant as possible without dislodging the bead pellet.

6.3 Wash

- Separate the magnet from the tubes or plate and add 400 µL of nuclease-free water.

  NOTE: Ensure that desalted or ultrapure water with a pH value below 7 is used.

- Vortex for 30 seconds and allow to rest for 30 seconds at room temperature.
- Bring the magnet into contact with the tube or plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Remove the supernatant and discard. Remove as much supernatant as possible without dislodging the bead pellet.

6.4 Elute

- Separate the magnet from the tubes or wells and add 100 µL of Elution buffer AMP.
- Vortex for 30 seconds, incubate 1 min at 60 °C and vortex for another 30 seconds.

  NOTE: Depending on the sample type and mixing mode, elution time and requirement for heated incubation may differ. In general, 1 minute elution time is sufficient, but very high molecular weight DNA samples may require prolonged heated incubation for efficient elution (5-10 minutes at 60 °C).

  NOTE: To obtain more highly concentrated DNA, elution buffer volume can be reduced to 20 µL.

- Bring the magnet into contact with the tube or plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Transfer the DNA eluate to a new tube or plate.

7. sbeadex Lightning Plant HTP DNA Kit protocol for DNA purification

The sbeadex Lightning Plant HTP Kit is specifically designed for high-throughput DNA purification from plant samples. The protocol is optimised for use in a 384-well plate format, providing a fast and cost-effective solution for genotyping workflows. However, it is also compatible with the 96-well plate format, offering flexibility based on sample throughput needs.

The following protocol outlines recommended processing volumes for the DNA purification step following homogenisation and lysis. These volumes are suitable for both 96-well and 384-well plate formats, allowing users to adapt the procedure to their specific sample types and workflow requirements.

7.1 Sample homogenisation and lysis

- Homogenise up to 30 mg fresh or up to 5 mg dried plant tissue (see section 4.8 for recommendations on homogenisation).
- Prepare a lysis solution by adding 2 µL Protease K solution (20 mg/mL) and 40 µL debris capture beads per mL of Lysis buffer PLA.

  NOTE: A premix of lysis buffer, Protease K solution, and debris capture beads is stable for 24 hours at room temperature.

- Add 200 µL of lysis solution to the homogenised sample and mix thoroughly.

NOTE: If fresh tissue has been used and Lysis buffer PLA was used during the homogenisation, no further addition of Lysis buffer PLA is required. Make sure to add 2 µL of Protease K solution (20 mg/mL) and 40 µL of debris capture beads per mL of Lysis buffer PLA before proceeding to the next step.

NOTE: Depending on the sample type and amount the lysis buffer volume may be adapted. Ensure that you can transfer 50 µL of cleared lysate without disturbing the pellet after the lysis.

- Incubate the sample at 55 °C for 30 minutes and mix thoroughly after the incubation.

  NOTE: Please see section 4.10 for recommendations on lysis buffer volumes.

  NOTE: Mixing or periodic mixing can be beneficial for an efficient lysis.

- After the incubation, centrifuge sample at maximum speed for 5 minutes to pellet the debris.

7.2 Bind

- Add 50 µL of Binding buffer LPL and 5 µL of sbeadex particle suspension to a fresh plate.

  NOTE: Ensure the sbeadex particle suspension is well mixed before use.
  NOTE: A pre-mix of Binding buffer LPL and sbeadex particle suspension is stable for at least one month at room temperature.

- Transfer 50 µL of the cleared lysate to the binding mix.
- Mix thoroughly to ensure proper resuspension of magnetic beads by shaking for at least 1 minute or pipetting up and down at least 10 times.

  NOTE: Efficient mixing affects binding and washing performance. For further recommendations on mixing refer to section 4.11.

  NOTE: If you encounter bead clumping while pipetting up and down consider using wide-bore tips.

- Bring the magnet into contact with the plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Remove the supernatant and discard. Remove as much supernatant as possible without dislodging the bead pellet.

7.3 Wash

- Separate the magnet from the plate and add 100 µL of nuclease free water.

  NOTE: Ensure that desalted or ultrapure water with a pH value below 7 is used.

- Mix thoroughly to ensure proper resuspension of magnetic beads by shaking for at least 1 minute or pipetting up and down at least 10 times.

  NOTE: Efficient mixing affects binding and washing performance. For further recommendations on mixing refer to section 4.11.

  NOTE: If you encounter bead clumping while pipetting up and down consider using wide-bore tips.
- Bring the magnet into contact with the plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Remove the supernatant and discard. Remove as much supernatant as possible without dislodging the bead pellet.

7.4 Elute

- Separate the magnet form the wells and add 50 µL of Elution buffer AMP
- Mix samples for at least 3 minutes on a shaker at 60-70 °C. Alternatively, mix by pipetting up and down at least 10 times to resuspend bead pellets and enable efficient DNA elution followed by a heated incubation at 60-70 °C for 2 minutes.

  NOTE: To obtain more highly concentrated DNA, elution buffer volume can be reduced to 20 µL.

  NOTE: Depending on the sample type and mixing mode, elution time and requirement to heated incubation may differ.

- Bring the magnet into contact with plate until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type).
- Transfer the eluates to a fresh plate.

8. Automation

Before transitioning to automation, we strongly recommend performing the sbeadex Lightning protocol manually to ensure it is fully optimised for your specific sample type. This step helps to establish a reliable baseline and allows for fine-tuning of critical parameters.

When automating the protocol, we advise starting with the same reagent volumes that proved to be effective during manual extraction. This approach supports consistency and facilitates troubleshooting during the initial automation phase.

Biosearch Technologies has decades of experience in automating nucleic acid extraction. Our R&D facilities are equipped with a wide range of automation platforms, and our team is ready to support your automation project with expert guidance. We are committed to helping you implement our chemistry in a way that aligns with your workflow and performance goals.

Additionally, we offer pilot studies and protocol customisation services to meet your specific requirements.

The sbeadex Lightning Kit is suitable to be automated on any liquid handler capable of handling magnetic bead-based kits as well as on magnetic rod automation platforms. In the following sections we provide some guidance for a selection of platforms.

8.1 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 purification.
Biosearch Technologies offers standard scripts for our oKtopure platform that can be downloaded here.

If you need support to implement the sbeadex Lightning Plant (HTP) DNA Kit on our oKtopure or further information on the system, please contact our technical support team (see section 10).

8.2 KingFisher Flex

For a flexible, medium-to-low throughput automation, the KingFisher™ Flex Purification System (ThermoFisher Scientific) can provide a sufficient solution. To streamline your workflow, we provide standard automation scripts for the sbeadex Lightning Plant (HTP) DNA Kit on the KingFisher Flex system. These support multiple elution time options, allowing easy adjustment for various sample types.

Thanks to the simplicity of the sbeadex Lightning chemistry, which requires only three positions on the KingFisher Flex system (Bind, Wash, Elute), our automation setup also enables high-throughput processing of 2×96 samples in a single run.

For specialised needs, Biosearch Technologies offers custom scripts with scaled-down buffer volumes upon request. Please contact our technical support team (see section 10) for assistance.

For standard KingFisher Flex scripts for both kits click on these links:

- KingFisher Flex scripts for sbeadex Lightning Plant DNA Kit
- KingFisher Flex scripts for sbeadex Lightning Plant HTP DNA Kit.

Each script is supplied together with a protocol report that provides additional details on buffer volumes and plate positions to support optimal setup and usage.

If protocol adjustments are necessary, please keep the following guidelines in mind:

1. Keep all volumes the same as for manual nucleic acid isolation. Longer elution times with heat might cause evaporation (e.g. for a 10-minute elution we recommend 20 µL additional elution buffer).
2. The incubation period for each bind and wash step should be a minimum of 1 minute to account for diffusion-dependent wash effects. Elution should be carried out at 70 °C for 1-10 minutes.
3. Prior to mixing for the washing and elution step, use the ‘Release Beads’ function with a ‘bottom mix’ for 10 seconds. Automated mixing should then be performed using the ‘Fast’ setting.

8.3 Other automation platforms

In general, the sbeadex Lightning Plant (HTP) 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 technical support team for guidance and support (see section 10).

9. Troubleshooting

Problem	Solution suggestion
Low yield	Multiple factors can affect low yield. Please see the list of solution suggestions below. **Homogenisation** Variable yields between samples may indicate incomplete homogenisation. • Visually check to ensure that the samples have been homogenised completely. • Look to optimise the homogenisation by modifying the time spent homogenising and the speed of the ball mill. The sample input amount can be adjusted and the state of sample prior to homogenisation could be changed (for example ensuring the sample is fully dry). See sections 4.7, 4.8, 4.9 and 4.10. **Lysis** Incomplete lysis may lead to low yields. • Check the lysis buffer for precipitation. See section 4.4 • Look to optimise the lysis by modifying the lysis time and temperature. • Visually check to ensure that the sample and lysis buffer are properly mixed. • Look to optimise the lysis buffer volume added to the sample to ensure complete lysis. • Ensure that the correct volume of lysate is transferred into the DNA binding reaction. When using multichannel pipettes or automating the process, pipette tips may clog due to viscous lysates or debris. In such cases, we recommend using wide-bore tips for the lysate transfer to prevent blockages and maintain consistency. • Certain sample types may require distinct lysis buffers. To support this, we offer the sbeadex Lightning Starter Kit, which includes six different lysis buffer options. This kit allows you to evaluate whether an alternative buffer improves lysis performance for your specific samples. **Elution** Incomplete DNA elution can lead to low yields. • Look to optimise the elution by increasing the elution time, increase the elution temperature and add a mixing step.
Low concentration	In addition to the factors relating to yield, the following can affect the final concentration. **Lysis** • Optimise the lysis buffer volume added to the sample. Reducing the lysis buffer volume can increase the final concentration. **Elution** • Optimise the elution volume to modify the final concentration. A higher elution buffer volume may result in increased DNA recovery for a higher total DNA yield. A lower elution buffer volume may result in decreased recovery but a higher final concentration.
Low purity	**Lysis** • Purity can be negatively affected by the transfer of sample plant material (debris) during the lysate transfer. Ensure clear lysate is transferred and optimise the lysis buffer volume if needed. • The sbeadex Lightning Starter Kit contains 6 different lysis buffer options. These alternative lysis buffers may deliver higher DNA quality, particularly better DNA purity, for your respective material-of-interest. **Binding and washing** • Make sure that the beads are fully resuspended during the binding and washing steps. Please refer to section 4.11 • Ensure that the supernatants of DNA binding and wash step are fully removed after the magnetic separation steps.

10. Further support

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

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

@LGCBiosearch | biosearchtech.com

BIOSEARCH™ TECHNOLOGIES