Manual

sbeadex Lightning nucleic acid purification chemistry

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BIOSEARCH™ TECHNOLOGIES

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Contents

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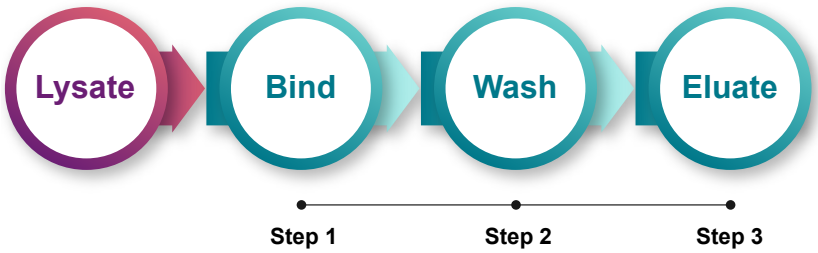
1. Purpose of this document

The purpose of this document is to provide a general introduction to sbeadex™ Lightning nucleic acid purification chemistry and universal details about how to run sbeadex Lightning in your laboratory. The document also contains links to specific laboratory protocols required to perform nucleic acid purification from different sample types, and information and guidance on the automation of protocols.

2. Introduction to sbeadex Lightning chemistry

sbeadex Lightning chemistry uses superparamagnetic microparticles and a novel one-step binding mechanism to bind and purify nucleic acids. Combined with a single water washing step, this unique process removes impurities and potential inhibitors of enzymatic reactions very effectively. Through this innovative technique, sbeadex Lightning supplies clean, automatable purifications at the speed of crude extraction methods.

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



The sbeadex Lightning nucleic acid purification chemistry is provided as a modular system. There are two core kits (A and B, see table 1), each containing a different binding buffer, sbeadex particles and the elution buffer. Alongside the core kits, a range of individual lysis buffers and supplements are offered by LGC Biosearch Technologies for the sample-specific protocols. The individual components can be purchased separately, to offer tailor-made solutions to our customers.

A sbeadex Lightning Nucleic Acid Purification Starter Kit is available to facilitate optimisation of sample lysis conditions for specific sample types. This consists of the binding buffers of both core kits, elution buffer and sbeadex particles as well as six different lysis buffers, Protease K solution and debris capture beads.

sbeadex Lightning kits	Description
sbeadex Lightning Nucleic Acid Purification Core Kit A	Suitable for the majority of sample types. Recommended for most plant samples
sbeadex Lightning Nucleic Acid Purification Core Kit B	Suitable for specific sample types especially mammalian tissue samples
sbeadex Lightning Nucleic Acid Purification Starter Kit	Suitable for all sample types and recommended to determine the optimal composition of core kit and single components

Table 1. sbeadex Lightning core kit and starter kit details. For detailed information regarding kit suitability for specific sample types, please see our website. Ordering information is also available on our website.

Biosearch Technologies do offer pilot studies and customised solutions where appropriate.

3. Kit contents and storage conditions

	Core Kit A			Core Kit B			
Component	NAP40-030-01	NAP40-030-02	NAP40-030-03	NAP40-031-01	NAP40-031-02	NAP40-031-03	Storage conditions
Binding buffer LP	22 mL	220 mL	2,200 mL	-	-	-	Room temperature*
Binding buffer LU	-	-	-	22 mL	220 mL	2,200 mL	Room temperature*
sbeadex particle suspension	2.2 mL	22 mL	220 mL	2.2 mL	22	220 mL	Room temperature
Elution buffer AMP	11 mL	110 mL	1,100 mL	11 mL	110	1,100 mL	Room temperature

Table 2. Components supplied in the sbeadex Lightning core kits. * store in the dark for long-term preservation

Component	Volume (mL)	Storage conditions
Binding buffer LP	2 mL	Room temperature; store in the dark for long-term preservation
Binding buffer LU	2 mL	Room temperature; store in the dark for long-term preservation
sbeadex particle suspension	0.4 mL	Room temperature
Elution buffer AMP	2 mL	Room temperature
Lysis buffer PN	4 mL	Room temperature
Lysis buffer PVP	4 mL	Room temperature
Lysis buffer UR	4 mL	Room temperature
Lysis buffer BL	4 mL	Room temperature
Lysis buffer H	4 mL	Room temperature
Lysis buffer LI	4 mL	Room temperature
Protease K solution	200 µL	Room temperature
Debris capture beads	160 µL	Room temperature

Table 3. Components supplied in the sbeadex Lightning Starter Kit.

	Part codes	Volume	Storage conditions
Lysis buffer PN	NAP10-006-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer PVP	NAP10-010-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer UR	NAP10-004-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer BL	NAP10-002-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer H	NAP10-003-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Lysis buffer LI	NAP10-005-00/...-01/...-02/...-03/...-04/...- 05	30-3,000 mL	Room temperature
Protease K solution	NAP30-002-01/...-02/...-03/...-04/...- 05/...-06	1-1,000 mL	Room temperature
RNase A solution	NAP30-003-01/...-02/...-03	1-100 mL	4 °C
Debris capture beads	NAP20-005-00/...-01/...-02/...-03/...-04/...- 05	1-1,000 mL	Room temperature

Table 4. Lysis buffers and supplements for sbeadex Lightning (purchased separately).

4. Experimental procedure

4.1 General information before starting

To ensure optimal results with the sbeadex Lightning chemistry, lysis buffer optimisation can increase yield and purity of the final nucleic acid eluate. The lysis buffers listed below have different chemical properties which allow for more optimal lysis depending on the sample material. Table 5 indicates which lysis buffer should be used for each sample type.

Lysis buffer PN	CTAB-based buffer – common plant lysis buffer
Lysis buffer PVP	CTAB-based buffer – for plant samples rich in polyphenols or samples rich in colour pigments (e.g. hair)
Lysis buffer BL	SDS-based lysis buffer
Lysis buffer H	Harsher SDS-based lysis buffer
Lysis buffer UR	CTAB-based lysis buffer – strong protein denaturation properties in conjunction with protease
Lysis buffer LI	SDS-based buffer – good performance on sample types such as seed, tissue, and young leaves

Table 5. The different lysis buffers available from Biosearch Technologies for use with sbeadex Lightning nucleic acid purification chemistry including guidance on suitability for sample types. Please note that sbeadex Lightning is also compatible with alternative lysis buffers; these can be trialled alongside the sbeadex Lightning core kits and our technical support team can provide support if required.

4.2 Lysis recommendations

Which lysis buffer to use and length of the lysis reaction depends on the sample material and the downstream needs. The appropriate lysis buffer must be determined empirically supported by the information in table 5. Recommendations for specific sample types can be accessed on our website.

4.2.1 Plant

Generally, plant material should be lysed for 10 to 60 minutes. For plant material, protease digestion is strongly recommended, using 2 µL Protease K solution (20 mg/mL) per mL lysis buffer. Protease K digestion should be performed at 55 °C. Premixes of Lysis buffers and Protease K solution are stable over the course of a day. For plant material with a lot of debris, 40 µL debris capture beads per mL lysis buffer can be added. If RNase digestion is required, around 1 µL RNase (20 mg/mL) per mL lysate can be added during lysis. If Protease K digestion is being performed, we recommend sequential digestion, first with RNase and then with Protease K solution. Alternatively, RNase digestion can be performed during the water wash step. All lysed samples should be centrifuged at maximum speed to pellet the debris. We suggest 2,500 x g for 10 minutes, although this will vary according to sample type. Following centrifugation, supernatant can then be transferred and used in the purification protocol.

4.2.2 Livestock

Livestock tissue benefits from an overnight lysis step with 10 µL Protease K solution (20 mg/mL) per 100 µL lysis reaction. Protease K digestion should be performed at 55 °C. Pre-mixes of lysis buffer and Protease K solution are stable over the course of a day. If RNA digestion is required, around 1 µL RNase A (20 mg/mL) per mL lysate can be added during lysis. If Protease K digestion is being performed, we recommend sequential digestion, first with RNase and then with Protease K solution. Alternatively, RNase digestion can be performed during the water wash step. If there is any debris remaining (e.g. hair) after lysis, centrifuge samples at maximum speed to pellet the debris and transfer supernatant for use in the purification protocol.

If samples are left submerged in conservation buffer during lysis (e.g. Allflex Tissue Sampling Units (TSUs) or Caisley DNA Tags), the standard protocol needs to be slightly adapted to achieve desired DNA yield. There are two possible options to optimise the lysis protocol for this sample type:
a) Prepare lysis mix: per 100 µL of Elution Buffer PN add 10 µL Protease K solution (20 mg/mL). Per 100 µL preservation solution add 110 µL of the previously prepared lysis mix. Continue with the standard DNA purification protocol.
b) Add one volume of Lysis buffer PVP with 10 µL Protease K solution (20 mg/mL) per 100 µL lysis reaction. If Lysis buffer PVP is used, you must replace the water wash step in the DNA purification protocol with Wash buffer TN2.

4.3 Optimising input volume

4.3.1 Plant samples

To ensure that the appropriate lysate volume is used, please consider the suggestions displayed in table 6. Since sample material can behave very differently depending on the species and treatment of the sample, the exact values required may differ. The supernatant row describes the expected volume of liquid that can be used for binding, after lysis of the different matrices with the displayed amount of lysis buffer.

	Leaf punch 3 mm		Leaf punch 6 mm		Ground (dried) seed		Ground (dried) leaf	
Sample	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**	**Fresh**	**Dried**
Lysis buffer [µL]	250	250	300	300	300	300	400	600
Supernatant [µL]	200	200	200	200	200	200	200	200

Table 6. Expected volumes of supernatant from lysis using different sample matrices and amounts. Please note that values may differ from plant to plant.

4.3.2 Livestock samples

For livestock tissue samples, the tissue should be submerged in lysis buffer. Volume of lysis buffer should be optimised depending on tissue type and nucleic acid content.

4.4 Magnets and alternatives

When performing the sbeadex 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.

4.5 Particle resuspension

It is important to ensure that the sbeadex particle suspension is properly re-suspended before adding it to the Binding buffer. 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.

4.6 Formation of precipitates in lysis buffers

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

4.7 Coloration of binding buffer and precipitates

Especially in binding buffer LP, to a lesser extend binding buffer LU, 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. Low amounts of precipitates will not affect performance. Shaking the binding buffers before usage is recommended.

4.8 Laboratory conditions

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

4.9 Required materials (not included)

The list below details the equipment and reagents that are required to perform sbeadex Lightning nucleic acid purification in your laboratory, in addition to the reagents supplied in the sbeadex Lightning Core Kit.

Essential

- Magnetic rack or centrifuge
- 96- or 384-well plates, or reaction tubes
- Centrifuge for plant samples
- Water bath or incubator (capable of temperatures up to 55 °C)
- Desalted or ultrapure water (pH below 7)

Recommended

- Protease K solution (20 mg/mL)
- Debris capture beads for plant leaf samples

Optional

- RNase A (20 mg/mL)
- Beta-Mercaptoethanol
- 1-Thioglycerol
- Tween 20
- Elution Buffer PN

4.10 Disruption and homogenisation of plant materials

Prior to performing the sbeadex Lightning nucleic acid purification protocol, each plant tissue sample must be homogenised. To obtain high molecular weight nucleic acids, it is recommended to lyophilise the plant material prior to homogenisation. Lyophilised plant tissue should be homogenised without the addition of lysis buffer. If fresh plant tissue is used (i.e. not lyophilised), it should either be snap frozen in liquid nitrogen prior to grinding, or homogenised after the addition of 250 µL lysis buffer. When processing small numbers of samples, plant tissue can be homogenised using one of the following methods:

- Using a mortar and pestle. Sea sand can be added to aid tissue disruption.
- In a 1.5 mL microcentrifuge tube, using a micro pestle.
- In a 1.5 mL/2 mL sample tube, using a steel ball and a ball mill.

When processing large numbers of samples, plant tissue can be homogenised using one of the following methods:

- In a rack of sample tubes e.g. 96 array of 1.4 mL tubes, using steel balls and a ball mill
- In a 96-well sealed plate, using steel balls and a ball mill.

5. Automation

Once the sbeadex Lightning purification protocol has been trialled manually (and optimised where necessary for your sample type), it is possible to automate the procedure to increase throughput. Biosearch Technologies recommends following the manual protocol 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 nucleic acid extraction specialists for technical support (see section 9). We also offer pilot studies and customised protocols where required.

For a flexible, medium-to-low throughput automation, the KingFisher™ Flex Purification System (ThermoFisher Scientific) can provide a sufficient solution. We provide standard protocols for the KingFisher (.bdz) on our website.

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. 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.

6. Protocols

Please visit the sbeadex Lightning resources page on our website to access the relevant protocol for your starting plant or livestock material.

7. Troubleshooting

Problem	Solution suggestion
Protocol is not fast enough	If the highest purity nucleic acids are not required for downstream applications, it is possible to omit the water wash step. Here, we recommend diluting the lysis buffers 1:1 (v/v) with 10 mM Tris pH 8 before using it for the lysis. Removing the binding mixture thoroughly before elution is crucial when applying this method. Increasing the elution buffer volume is also recommended when omitting the water wash step.
Beads take longer than expected to pellet using a magnet	The rate of bead pelleting when using a magnet will vary depending on the sample type and magnet strength. An optional short spin before magnetising will help beads to pellet more quickly.
Low 260/280 or 260/230 measurements	Ratios depend on the lysis buffer used. If none of the lysis buffers show good or acceptable ratios, there might be a sample overload. → Try using less material or more lysis buffer. If the 260/280 ratio is low (below 1.7): → prolonged lysis time or increasing amount of Protease in the lysis buffer might help → add 0.1 % (v/v) Tween 20 to the elution buffer.
Low nucleic acid yield	Yields depend on the lysis buffer used. If all lysis buffers show low yield and Protease was added: → prolong the elution time and/or the lysis time → use heated elution buffer or heat during elution → add 0.1 % (v/v) Tween 20 to the elution buffer → use less material or more lysis buffer. In rare cases, the water wash might wash away some nucleic acid → use Wash buffer PN2 instead of the water wash.
Poor downstream results	→ Make sure that no solid plant material from the lysate is transferred to the purification step. → In case of cloudy eluates, it might be possible to spin samples down to obtain clear eluates. → The choice of lysis buffer determines the potential presence of secondary metabolites in eluates.
High molecular weight nucleic acid does not elute sufficiently	To obtain more high molecular weight nucleic acid a longer elution time (e.g. 10 minutes) may increase yield.

8. Frequently asked questions

Please visit our website to explore our online FAQs.

9. Further support

If you require any further support for any of the sbeadex products, please contact our technical support team at techsupport@lgcgroup.com or submit a request for support directly into our case system.

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