Manual

General sbeadex plant manual

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BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

General sbeadex plant manual

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Manual

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1. Purpose of this document

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

If you are new to sbeadex, this document will provide all the information that you need to get started with sbeadex in your laboratory.

sbeadex Plant DNA Purification Kits can be used for a variety of plant samples. For a list of currently tested sample types, please see our website.

2. Introduction to sbeadex chemistry

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. Figure 2.1 illustrates the novel two-step binding mechanism employed by the sbeadex chemistry.

The absence of any organic solvents in the final wash buffer(s) prevents the nucleic acid preparation from being contaminated with inhibitory remains of these solvents. The overall purification time is also shortened as the drying and heating steps typically required with organic solvents are not necessary. The sbeadex chemistry delivers nucleic acids of high yield, purity and quality that is suited for many downstream applications such as PCR or sequencing/NGS.

LGC Biosearch Technologies manufactures a range of different sbeadex nucleic acid purification kits that are suitable for use with a broad range of starting materials. Kit types include, but are not limited to, sbeadex livestock, sbeadex plant, sbeadex blood, sbeadex tissue, sbeadex plasmid and sbeadex pathogen. The reagents in these individual kits have been optimised to facilitate efficient and reliable purification from the appropriate starting materials. In addition, the sbeadex chemistry is perfectly adapted to our high-throughput nucleic acid isolation platform, the oKtopure™, and is also suitable for many popular robotic platforms that are able to manipulate paramagnetic beads (see section 3.9).

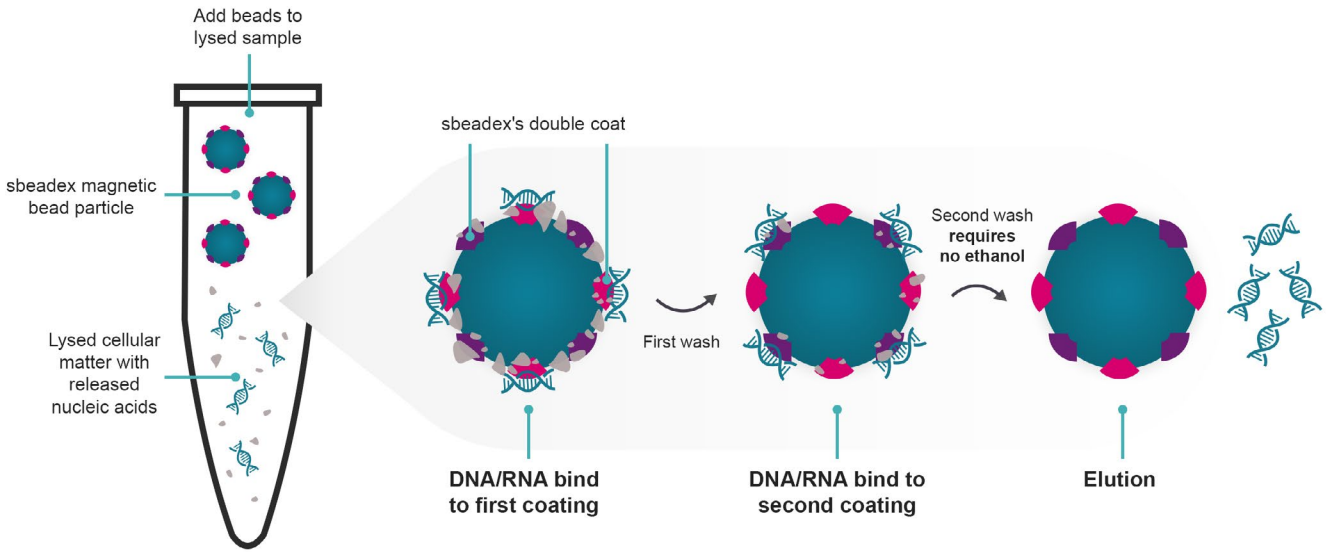

Figure 2.1. A schematic of the sbeadex novel two-step binding mechanism.

Even though the sbeadex standard protocols work with many different plant and livestock sample materials, there can be considerable differences in sample quality due to factors including age, storage conditions, and origin. Performing an initial pilot study and developing a tailored protocol is sometimes an option to provide users a ready-to-go solution.

If required, Biosearch Technologies can provide pilot studies to develop the optimal protocol for the respective sample material. We also offer customised solutions or kits that are developed and optimised for specific sample materials and customer requirements. Please contact our technical support for more information about these options (see section 4).

For ordering information please refer to our website.

3. Experimental procedure

3.1 General information before starting

If performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds (single tubes: full speed; plates: 2000 × g) to enable the magnetic particles to form a pellet.

The technical definitions and considerations detailed in table 3.1 should be applied to the experimental process each time they are stated.

All sbeadex plant buffers are stable at room temperature and can be stored accordingly. As Elution buffer AMP, Elution buffer PN, sbeadex particles and Wash buffer PN2 do not contain any substances that inhibit microbial growth, it can be beneficial to store these at 4 °C after opening if contamination of these buffers pose a risk in your working environment.

Protocol process	
Bring magnetic rack into contact with tubes	This will allow the sbeadex beads to form a pellet on the side of the tube, to allow for easy removal of the supernatant. The times stated for sbeadex bead pelleting are minimum recommended incubation times. The strength of the magnetic rack will influence the speed of sbeadex beads pelleting. If required, increasing incubation time should be used to ensure all beads are pelleted.
Mix thoroughly	The sample should be mixed thoroughly (preferably using a shaker), to ensure the sbeadex beads are completely resuspended. The mixing can be assisted by periodic vortexing in 5-10 second bursts.
Removal of supernatant	When removing supernatant, it is important to remove as much liquid as possible without dislodging the particle pellet. To avoid disruption of the particle pellet when placing the pipette tip inside the tube, ensure that the tip is aimed towards the sample tube wall opposite the 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.
Constant shaking	The sample should be constantly agitated by vortexing/shaking to ensure the sbeadex beads do not settle. This movement will increase the efficiency of the binding and washing steps.

Table 3.1. Technical descriptions for the protocols outlined in this manual and the considerations which should be adhered to when following the methods.

3.2 Magnets and alternatives

When performing the sbeadex kit protocol, a magnet or centrifuge is required to pellet the magnetic particles. If performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds (single tubes: full speed; plates: 2000 × g) to enable the magnetic particles to form a pellet.

3.3 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.

3.4 Formation of precipitates in buffers

Salt precipitates can form in Lysis buffer 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 thoroughly to re-dissolve the precipitates.

3.5 Laboratory conditions

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

3.6 Required materials (not included)

The list below details the equipment and reagents that are required to perform sbeadex purification in your laboratory, in addition to the reagents supplied in a sbeadex kit.

Essential

- Magnetic rack or centrifuge
- 96- or 384-well plates, or reaction tubes
- Centrifuge
- Water bath or incubator (capable of temperatures up to 55 °C)

Optional or required only for specific sbeadex plant kits

- RNase A*
- Protease K*
- Debris capture beads*
- Propan1-ol
- DNA/RNA carrier
- Mercaptoethanol
- 1-Thioglycerol

*can be included in custom kits

3.7 Disruption and homogenisation of plant material

Prior to performing the sbeadex plant DNA purification protocol, each plant tissue sample must be homogenised. To obtain high molecular weight DNA, 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.

3.8 Optimising input volume

To ensure that an appropriate lysate volume is used for your samples, please consider the suggestions displayed in table 3.2. Sample material can behave very differently depending on the species and treatment of the sample, therefore the exact values required may differ. In table 3.2 the supernatant row details the expected volume of liquid available for subsequent binding steps, after performing lysis of the different matrices with the indicated volume of lysis buffer.

	Leaf punch 3 mm		Leaf punch 6 mm		Ground (dried) seed		Ground (dried) leaf	
Sample	Fresh	Dried	Fresh	Dried	15 mg	30 mg	15 mg	30 mg
Lysis buffer (µL)	100	100	150	150	150	300	150	300
Supernatant (µL)	80	80	100	100	100	200	40	80

Table 3.2. Expected volumes of supernatant from lysis using different sample matrices and amounts. Please note that values might differ from plant to plant.

3.9 Automation

Once the sbeadex DNA extraction 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 4). 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. In our kit-specific protocols (section 3.10), Biosearch Technologies provide KingFisher BindIt files for the automation of each kit.

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.

3.10 Protocols

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

3.11 Frequently asked questions

Please visit our website to explore our online FAQs.

4. 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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