sbeadex Mini Plant DNA Purification Kit (trial kit) protocol

Catalogue number: NAP41606 and NAP41619

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Not for use in diagnostic procedures.

1. Purpose of this document

This document contains the laboratory protocol for the sbeadex™ Mini Plant DNA Purification Kit (trial kit) and provides specific guidance for automation of the DNA purification process. The sbeadex Mini Plant DNA Purification Kit (trial kit) is supplied to enable users to test the performance of sbeadex DNA purification chemistry in their laboratory, and to provide the option to develop a protocol that is optimised for their specific plant leaf or seed material.

As a guide, 10 µL of sbeadex particle suspension (standard mini scale extraction) has the capacity to purify approximately 2 µg high molecular weight DNA. Final yield and quality of DNA will depend on the degree of sample lysis, storage of sample prior to extraction, and genome size as well as a range of other parameters.

Our experienced nucleic acid purification team is happy to answer any questions that you may have about using the sbeadex plant trial kit in your laboratory and are always available to discuss possible protocol optimisation with users (see section 4 for contact details).

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2. Kit contents and storage conditions

Please note: This kit contains three different lysis buffers (P/PVP/BL). The user should select the most appropriate lysis buffer for their plant tissue type prior to performing the protocol (see section 3.1).

Kit component	Volume supplied per product code		Storage conditions
	NAP41606 (96 preparations)	NAP41619* (96 preparations)	
Lysis buffer PN	15 mL	15 mL	Room temperature
Lysis buffer BL	15 mL	15 mL	Room temperature
Lysis buffer PVP	15 mL	15 mL	Room temperature
Binding buffer PN	15 mL	-	Room temperature
Binding buffer PN (concentrate)	-	7.5 mL	Room temperature
sbeadex particle suspension	1 mL	1 mL	Room temperature
Wash buffer PN1	30 mL	-	Room temperature
Wash buffer PN1 (concentrate)	-	20 mL	Room temperature
Wash buffer PN2	30 mL	30 mL	Room temperature
Elution buffer PN	15 mL	15 mL	Room temperature
Protease K solution	0.1 mL	0.1 mL	Room temperature
Elution buffer AMP	15 mL	15 mL	Room temperature

Table 1. Components supplied in the sbeadex Mini Plant DNA Purification Kit (trial kit).
* Denotes no dangerous goods part code. Sufficient concentrate supplied to make up to stated volume as indicated on the bottle label.

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3. sbeadex plant trial kit protocol

3.1. Choosing the appropriate lysis buffer

To obtain a high quantity and quality of purified DNA, it is important to select the appropriate lysis buffer for your sample type. The sbeadex Mini Plant DNA Purification Kit (trial kit) includes three different lysis buffers that provide options for a wide range of plant tissue types.

If you are using leaf tissue as starting material, please use table 2 to determine the recommended lysis buffer. If you are using seed tissue as starting material, please use table 3 to determine the recommended lysis buffer.

If your plant species is not listed, we recommend identifying a species that is genetically similar to your species in the tables and starting with that buffer. You can always then try one of the alternative buffers to see if this improves protocol performance for your species.

Leaf tissue

Plant species	Recommended lysis buffer
Apple, aubergine (eggplant), barley, canola, chilli, corn, flax, juniper, okra, onion, pepper, potato, rice, salad, sugar cane, sunflower, sweetcorn, tobacco, tomato, watermelon, wheat	Lysis buffer PN
Aegilops sharonensis, Brachypodium distachyon, Nicotiana paniculata	Lysis buffer PN + Thioglycerol
Apricot, cocoa, cotton, peach, watermelon, sunflower	Lysis buffer PVP

Table 2. Recommended lysis buffers for leaf tissue purifications using sbeadex chemistry.

Seed tissue

Plant species	Recommended lysis buffer
Barley, sugar beet, soy	Lysis buffer PN
Aubergine (eggplant), canola, chilli, corn, cucumber, melon, okra, onion, pepper, tomato, wheat	Lysis buffer BL + Protease K solution

Table 3. Recommended lysis buffers for seed tissue purifications using sbeadex chemistry.

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3.2. Before you start

- This protocol assumes that 20-30 mg of fresh plant tissue will be used per sample. If a larger quantity of tissue is to be used, a greater volume of lysis buffer will be required. 50 µL of lysate is required for continuation of the extraction protocol after tissue homogenisation and lysis.
- Tough plant material (e.g. monocot leaf tissue) can be frozen using dry ice or liquid nitrogen prior to the addition of lysis buffer, to aid the tissue grinding process.
- If thioglycerol is required, this should be added to the appropriate lysis buffer to give a final concentration of 1%. Thiogyclerol can be replaced by β-mercaptoethanol or 10 mM DTT.
- If Protease K solution is required, this should be added to the appropriate lysis buffer to give a final concentration of 2 µL/mL (stock solution provided at 20 mg/mL).
- Binding buffer PN and sbeadex particle suspension can be premixed in advance and stored at room temperature for 2 weeks if desired. This should be prepared as detailed in step 1 of section 3.3.

3.3. Performing the protocol manually

Before commencing the purification protocol, ensure that plant tissue samples have been appropriately homogenised as detailed in section 3.7 of the sbeadex plant manual and the general information stated in section 3.2 (above) has been considered.

1. Incubate homogenised tissue in the selected lysis buffer at 65 ºC for 10-60 minutes to lyse cells.
   - **Please note:** Seed powder/flour may soak up all the lysis buffer during this step. Please add additional lysis buffer if this occurs as 50 µL of lysate is required for continuation of the protocol.
2. Centrifuge lysed samples at 2500 × g for 10 minutes to pellet the debris.
3. Prepare the binding solution in a fresh tube - one for each tissue sample to be purified:
   - In the fresh tube, add 120 µL Binding buffer PN 🟢.
   - Add 10 µL of sbeadex particle suspension ◯ to each tube (Please note: ensure that the magnetic particles within the sbeadex particle suspension are fully re-suspended before transferring).
4. Transfer 50 µL of lysate (from the lysis performed in steps 1 and 2) to each tube of binding solution (from step 3). Set the pipette to 150 µL and pipette up and down five times to ensure that lysate and binding solution are thoroughly mixed.
5. Incubate at room temperature for 4 minutes to allow binding to occur.
6. Bring a magnet into contact with the sample tube(s) at room temperature for 1 minute. This will allow the sbeadex particles to form a pellet (Please note: If a magnet is not available, it is recommended to spin the tubes for 10 seconds at 2500 × g to pellet the sbeadex particles).

7. Before moving the magnet away from the sample tube(s), remove the supernatant and discard. Ensure that as much of the supernatant is removed as possible without dislodging the sbeadex particle pellet.
8. Move the magnet away from the sample tubes.
9. Add 200 µL of Wash buffer PN1 🔴 to each tube. Mix thoroughly by agitation, then set the pipette to 150 µL and pipette up and down five times to ensure that pellet is fully resuspended.
10. Incubate at room temperature for 10 minutes, agitating the sample regularly using a shaker or a vortex.
11. Bring a magnet into contact with the sample tube(s) at room temperature for 1 minute. This will allow the sbeadex particles to form a pellet (Please note: If a magnet is not available, it is recommended to spin the tubes for 10 seconds at 2500 × g to pellet the sbeadex particles).
12. Before moving the magnet away from the sample tube(s), remove the supernatant and discard as before, ensuring as much of the supernatant is removed as possible without dislodging the sbeadex particle pellet.
13. Move the magnet away from the sample tubes.
14. Add 200 µL of Wash buffer PN2 🟡 to each tube. Mix thoroughly by agitation, then set the pipette to 150 µL and pipette up and down five times to ensure that pellet is fully resuspended.
15. Repeat steps 10 to 13.
16. Add 200 µL pure water to each tube. Mix thoroughly by agitation, then set the pipette to 150 µL and pipette up and down five times to ensure that pellet is fully resuspended.
17. Repeat steps 10 to 13.
18. Add 70 µL Elution buffer ⚫ to each tube. Mix thoroughly by agitation, then set the pipette to 65 µL and pipette up and down five times to ensure that pellet is fully resuspended.
    - Please note: Elution buffer AMP is the preferred buffer for elution at room temperature. Biosearch Technologies recommends testing the performance of Elution buffer AMP in your downstream processes first before using this for all of your samples. In case of unsatisfactory results, the use of Elution buffer PN is recommended.
19. Incubate at room temperature for 10 minutes, agitating the sample regularly using a shaker or a vortex.
20. Bring a magnet into contact with the sample tube(s) at room temperature for 3 minutes. This will allow the sbeadex particles to form a pellet (Please note: If a magnet is not available, it is recommended to spin the tubes for 10 seconds at 2500 × g to pellet the sbeadex particles).
21. Remove the eluate by pipetting, and transfer into a fresh tube. To avoid sbeadex particle transfer it is recommended to transfer only 50 µL of the eluate.

3.4. Automating the protocol

3.4.1 Automation via the KingFisher Flex Purification System

Biosearch Technologies provides a KingFisher™ BindIt file for the automation of the sbeadex Mini Plant DNA Purification Kit (trial kit) on the KingFisher Flex Purification System (ThermoFisher Scientific).

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

1. Keep all volumes the same as for manual nucleic acid isolation, 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 18 of the manual protocol; see above).
2. The incubation period for each bind and wash step should be a minimum of 5 minutes 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.

3.4.2 Automation via the oKtopure

The sbeadex Mini Plant DNA Purification Kit (trial kit) protocol can be automated using our fully automated nucleic acid isolation platform, the oKtopure™. Please visit our website for more details.

To enquire about the oKtopure or to discuss a pilot study, please contact your local sales representative.

5. Further support

If you require any further support 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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