sbeadex Mini Plant DNA Purification Kit protocol

Catalogue numbers: NAP41601, NAP41610 and NAP41618

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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 and provides specific guidance for automation of the DNA purification process. The sbeadex Mini Plant DNA Purification Kit is used to isolate DNA from a wide variety of plant species and is optimised for extraction from both fresh and lyophilised plant tissue material including leaves and roots. Typical quantities of starting material are 5-10 mg lyophilised tissue or 10-30 mg fresh tissue.

2. Kit contents and storage conditions

Kit component	Volume supplied per product code			Storage conditions
	NAP41601 (96 preparations)	NAP41610 (960 preparations)	NAP41618* (960 preparations)	
Lysis buffer PN	15 mL	100 mL	100 mL	Room temperature
Binding buffer PN	15 mL	200 mL		Room temperature
sbeadex particle suspension	1.1 mL	11 mL	11 mL	Room temperature
Wash buffer PN1	30 mL	200 mL		Room temperature
Wash buffer PN2	30 mL	200 mL	200 mL	Room temperature
Elution buffer PN	15 mL	100 mL		Room temperature
Binding buffer PN (concentrate)			100 mL	Room temperature
Wash buffer PN1 (concentrate)			134 mL	Room temperature
Elution buffer AMP			100 mL	Room temperature

Table 1. Components supplied in the sbeadex Mini Plant DNA Purification 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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sbeadex Mini Plant DNA Purification Kit

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3. sbeadex Mini Plant DNA Purification Kit protocol

3.1. 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 has been considered.

1. Add 90 µL Lysis buffer PN ● to each homogenised sample. Please note: If fresh tissue has been used, and Lysis buffer PN was used in the homogenisation, no further addition of Lysis buffer PN is required.
   If RNase A is being used, it should be added during this step.
2. Incubate at 65 °C for a minimum of 10 minutes.
3. Centrifuge at 2500 x g for 10 minutes to pellet the debris. The supernatant in this tube is referred to as the lysate.
4. Add 120 µL Binding buffer PN ● and 10 µL sbeadex particle suspension ○ to a fresh sample tube. Please note: Ensure that the sbeadex particle suspension is mixed well before use.
5. Transfer 50 µL lysate (from step 3) into the tube prepared in step 4 (i.e. containing Binding buffer PN and sbeadex particle suspension). Mix thoroughly by setting the pipette volume to 150 µL and pipetting up and down five times.
6. Incubate at room temperature for 4 minutes to allow binding to occur.
7. Bring magnet into contact with the tube(s) for 1 minute at room temperature. sbeadex particles will form a pellet.
8. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible and take care not to dislodge the pellet.
9. Separate the magnet from the sample tubes.
10. Add 200 µL Wash buffer PN1 ● to the tube containing the pellet. Mix thoroughly by pipetting (suggested pipette volume 150 µL) to fully re-suspend the pellet.
11. Incubate at room temperature for 10 minutes. Periodically agitate the sample using a shaker or vortexer.
12. Bring magnet into contact with the tube(s) for 1 minute at room temperature. sbeadex particles will form a pellet.
13. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible and take care not to dislodge the pellet.
14. Separate the magnet from the sample tubes.
15. Repeat steps 10 to 14 with 200 µL Wash buffer PN2 ●.
16. Repeat steps 10 to 14 with 200 µL sterile ultrapure water.
17. Add 70 µL Elution buffer PN ● to the pellet. Mix thoroughly by pipetting (suggested pipette volume 65 µL), ensuring that the pellet is fully re-suspended.

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18. Incubate at 55 °C for 10 minutes. Periodically agitate the sample using a heated shaker or vortexer.
    NOTE: Elution can be performed at room temperature if required. A 20% reduction in DNA yield is typically observed when elution is performed at room temperature. An elution temperature of 55 °C is recommended to maximise DNA yield.
19. Bring magnet into contact with the tube(s) for 3 minutes at room temperature. sbeadex particles will form a pellet.
20. Transfer the eluate to a new tube by pipetting. To avoid particle transfer it is recommended to transfer only 50 µL of the eluate.

2.2. Automating the protocol

2.2.1 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 on the KingFisher Flex Purification System (Thermo Fisher 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 17 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.

2.2.2 Automation via the oKtopure

The sbeadex Mini Plant DNA Purification 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.

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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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