sbeadex Maxi Plant DNA Purification Kit protocol

Catalogue number: NAP41602, NAP41620, NAP41624 and NAP41623

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

1. Purpose of this document

This document contains the laboratory protocol for the sbeadex™ Maxi Plant DNA Purification Kit and provides specific guidance for automation of the DNA purification process. The sbeadex Maxi Plant DNA Purification Kit is suitable for DNA isolation from a wide variety of plant species and is optimised for purification from both fresh and lyophilised plant tissue material including leaves and roots. Typical quantities of starting material for the 'maxi kit' are 10-30 mg lyophilised tissue or 40-120 mg fresh tissue.

2. Kit contents and storage conditions

	Volume supplied per product code				
Kit component	**NAP41602** (96 preparations)	**NAP41620** (960 preparations)	**NAP41624*** (96 preparations)	**NAP41623*** (960 preparations)	**Storage conditions**
Lysis buffer PN	30 mL	250 mL	30 mL	250 mL	Room temperature
Binding buffer PN	60 mL	600 mL			Room temperature
sbeadex particle suspension	6.5 mL	60 mL	6.5 mL	60 mL	Room temperature
Wash buffer PN1	60 mL	500 mL			Room temperature
Wash buffer PN2	60 mL	500 mL	60 mL	500 mL	Room temperature
Elution buffer PN	15 mL	100 mL	15 mL	100 mL	Room temperature
Binding buffer PN (concentrate)			30 mL	300 mL	Room temperature
Wash buffer PN1 (concentrate)			40.2 mL	335 mL	Room temperature

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

Protocol

3. sbeadex Maxi 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 250 µL Lysis buffer PN to each homogenised sample.
   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.
   NOTE: If RNase A is being used, it should be added during this step.
2. Incubate at 65 °C for >10 minutes.
3. Centrifuge at 2500 × g for 10 minutes to pellet the debris. The supernatant in this tube is referred to as the lysate.
4. Add 520 µL Binding buffer PN and 60 µL sbeadex particle suspension to a fresh sample tube.
   NOTE: Ensure that the sbeadex particle suspension is mixed well before use.
5. Transfer 200 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 700 µL and pipetting up and down 5 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 400 µL Wash buffer PN1 . Mix thoroughly by pipetting (suggested pipette volume 350 µL) to fully re-suspend the pellet.
11. Incubate at room temperature for 10 minutes. Periodically agitate the sample using a shaker or vortex.
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 400 µL Wash buffer PN2 .
16. Repeat steps 10 to 14 with 400 µL sterile, ultrapure water.

(Protocol continues on next page)

17. Add 100 µL Elution buffer PN ● to the pellet. Mix thoroughly by pipetting (suggested pipette volume 75 µL), ensuring that the pellet is fully re-suspended.
18. Incubate at 55 °C for 10 minutes. Periodically agitate the sample using a heated shaker or vortex. 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 80 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 Maxi 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 Maxi 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.

sbeadex Maxi Plant DNA Purification Kit

Protocol

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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