sbeadex Lightning protocol for plant and livestock tissue DNA purification

Protocol

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

1. Purpose of this document

This document contains the laboratory protocol for the sbeadex™ Lightning nucleic acid purification chemistry and provides specific guidance for automation of the DNA purification process. This sbeadex Lightning protocol is suitable for DNA isolation from plant and livestock tissue from a wide variety of species and is optimised for purification from both fresh and lyophilised tissue. For plant samples, typical quantities of starting material are 10-30 mg of lyophilised tissue or 40-120 mg of fresh tissue per purification. For livestock, quantities of starting material will vary depending on sample type.

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Protocol

2. sbeadex Lightning protocol

2.1. Performing the protocol manually

Before commencing the purification protocol, ensure that tissue samples have been appropriately homogenised, lysed and centrifuged as detailed in sections 4.2 and 4.10 of the sbeadex Lightning manual and the general information stated in section 4.1 has been considered.

1. Add appropriate amounts of Binding buffer LP or LU and sbeadex particle suspension to lysed samples. This can be done by either:
   a. preparing a binding mix by combining Binding buffer LP or LU and sbeadex particle suspension, and then adding this mix to the lysate. Sufficient volume of binding mix for all samples can be prepared in advance of commencing the protocol and is stable for at least one month.
   b. adding sbeadex particle suspension to the lysate, followed by Binding buffer LP or LU. If adding components separately, ensure sbeadex particle suspension is always added before the Binding buffer.

   Calculating volumes required

   The volume of Binding buffer required per sample is equal to the amount of lysate to be processed. For every 100 µL of Binding buffer, 10 µL of sbeadex particle suspension is required.

   For example, for 200 µL of lysate, a total of 220 µL of binding mix (as prepared in 1a) should be added, consisting of 200 µL binding buffer + 20 µL sbeadex particle suspension. If adding components separately, 20 µL of sbeadex particle suspension should be added to the lysate, followed by 200 µL Binding buffer for 200 µL of lysate.

   NOTE: Ensure that the sbeadex particle suspension is mixed well before use.

2. Vortex for 30 seconds and allow to rest for approximately 30 seconds at room temperature.

   NOTE: Since vortexing is critical at this step, ensure the samples are thoroughly mixed.

3. Bring magnet into contact with the tube(s) until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type). Ensure that all sbeadex particles are pelleted before proceeding to step 4.
4. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible and take care not to dislodge the pellet.
5. Separate the magnet from the tube(s).
6. Add the appropriate volume of water and vortex for 30 seconds. Allow to rest for 30 seconds at room temperature. Water volume should be double the volume of lysate used in step 1 (e.g. if 200 µL lysate was used, add 400 µL water). Allow to rest for 30 seconds at room temperature.

7. Bring magnet into contact with the tube(s) until all sbeadex particles form a pellet (usually 15-60 seconds depending on sample type). Ensure that all sbeadex particles are pelleted before proceeding to step 8.
8. Remove the supernatant and discard. Ensure that as much supernatant is removed as possible and take care not to dislodge the pellet.
9. Add the appropriate volume of Elution buffer AMP and vortex for 60 seconds. Alternatively, vortex for 30 seconds and incubate at 60 °C for 1-5 minutes. Elution buffer AMP volume should be 0.5x the volume of lysate used in step 1 (e.g. if 200 µL lysate was used, add 100 µL Elution buffer AMP). To obtain higher concentrated DNA, elution buffer volume can be reduced to 20 µL.

   NOTE: Depending on the sample type and mixing mode, elution time and requirement to heat may differ. In general, 1 minute elution time is sufficient but high molecular weight DNA samples can take longer to elute.

10. Bring magnet into contact with the tube(s) until sbeadex particles form a pellet (usually 20-60 seconds).
11. Transfer the eluate to a new tube by pipetting.

2.2 Automating the protocol

2.2.1 Automation via the KingFisher Flex Purification System

LGC Biosearch Technologies provide a KingFisher™ BindIt file for the automation of the sbeadex Lightning DNA purification protocol 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. Longer elution times with heat might cause evaporation (e.g. for a 10 minute elution we recommend 20 µL additional elution buffer).
2. The incubation period for each bind and wash step should be a minimum of 1 minute to account for diffusion-dependent wash effects. Elution should be carried out at 70 °C for 1-10 minutes.
3. Prior to mixing for the washing and elution step, 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 Lightning DNA purification protocol can be automated using our fully automated nucleic acid isolation platform, the oKtopure™ platform. Please visit our website for more details.

To enquire about the oKtopure or to discuss a pilot study, please contact us using the details below.

6. 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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