sbeadex maxi plant kit

Catalogue numbers 41602 and 41620
(For research use only. Not for use in diagnostic procedures.)

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1. Introduction

The sbeadex™ maxi plant kit is used to extract 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 for the maxi kit are 10-30 mg lyophilised tissue or 40-120 mg fresh tissue. Examples of plant species that have been successfully extracted using this kit include:

• Tomato
• Pepper
• Lettuce
• Maize
• Sunflower
• Rapeseed

Please contact our technical support specialists for details of other plant species that have been successfully extracted using this kit (see Section 7 for contact details).

Using this kit, high quality DNA can be extracted that is suitable for use in all downstream applications including PCR-based protocols and next generation sequencing.

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. The absence of any organic solvents in the final wash buffers prevents your nucleic acid preparation from being contaminated with inhibitory remains of these solvents and shortens the overall extraction time due to unnecessary drying and heating steps.

2. Kit contents and storage conditions

2.1 sbeadex maxi plant kit contents and storage conditions

The kit contents and storage conditions are outlined in Table 1 below (NAP41602 is sufficient for 96 extractions and NAP41620 is sufficient for 960 extractions). All kit components should be used within 12 months of receipt. Please refer to the kit box label for the expiry date.

<table>
<thead>
<tr>
<th>Component</th>
<th>Label colour</th>
<th>Volume (NAP41602)</th>
<th>Volume (NAP41620)</th>
<th>Storage conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis buffer PN</td>
<td>Blue</td>
<td>30 mL</td>
<td>250 mL</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Binding buffer PN</td>
<td>Green</td>
<td>60 mL</td>
<td>600 mL</td>
<td>Room temperature</td>
</tr>
<tr>
<td>sbeadex particle suspension</td>
<td>White</td>
<td>6.5 mL</td>
<td>60 mL</td>
<td>Room temperature (4 °C after opening)</td>
</tr>
<tr>
<td>Wash buffer PN1</td>
<td>Red</td>
<td>60 mL</td>
<td>500 mL</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Wash buffer PN2</td>
<td>Yellow</td>
<td>60 mL</td>
<td>500 mL</td>
<td>Room temperature (4 °C after opening)</td>
</tr>
<tr>
<td>Elution buffer PN</td>
<td>Black</td>
<td>15 mL</td>
<td>100 mL</td>
<td>Room temperature (4 °C after opening)</td>
</tr>
</tbody>
</table>

Table 1. sbeadex maxi plant kit contents including volumes and required storage conditions.
Additional buffers can be purchased separately if required (catalogue numbers available on request). Please note: Ultrapure sterile water is required for the wash steps of the protocol. This is not provided in the kit.

2.2 Formation of precipitates in buffers
Salt precipitates can form in Lysis buffer PN 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 stir thoroughly to re-dissolve the precipitates.

2.3 Additional reagents that may be required
Depending on the sample type, some protocols may require additional reagents for optimal DNA extraction.

- **2-mercaptoethanol or thioglycerol**
  For plant material that contains polyphenolic compounds, it is recommended that 1 % 2-mercaptoethanol or 1 % thioglycerol is added to Lysis buffer PN. Buffer that has had either of these reducing agents added to it must be used immediately after preparation.

- **Digestion with RNase A**
  sbadex particles co-isolate RNA during the extraction procedure. The presence of RNA in extracted samples can interfere with certain downstream processes such as UV measurement or enzymatic processing of genomic DNA. It is recommended to digest RNA with RNase A during the lysis step. 1 unit of RNase A (supplied from LGC at 7000 U per mL, use 0.15 µL per sample) should be added to each sample after homogenisation (i.e. at Step 1 of the laboratory procedure).

Ultrapure sterile water is also required during the wash steps of the sbadex maxi plant kit protocol.

3. Experimental procedure

3.1 Performing the protocol manually
When performing the sbadex maxi plant kit DNA extraction protocol, a magnet or centrifuge is required to pellet the magnetic particles.

When removing supernatants, it is important to remove as much liquid as possible without dislodging the particle pellet. When placing the pipette tip inside the tube, ensure that the tip is aimed towards the front wall of the sample tube to avoid disruption of the particle 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.

After the addition of each wash buffer, ensure the pellet is homogenised thoroughly in the wash buffer (e.g. by vortexing or pipetting up and down) to facilitate the best possible washing of the DNA. If performing the protocol manually without access to a magnet, sample tubes can be centrifuged for 10 seconds to enable the magnetic particles to form a pellet.

For information on automation of the sbadex maxi plant DNA extraction protocol, see Section 6 of this manual.
3.2 Before you start

3.2.1. Disruption and homogenisation of plant material

Prior to performing the sbeadex maxi plant DNA extraction 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 PN.

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.

3.2.2. Disruption and homogenisation of plant material

- sbeadex particle suspension
  Mix the suspension thoroughly before use to fully re-suspend the particles.

3.3. Laboratory procedure

Before commencing the extraction protocol, ensure that plant tissue samples have been appropriately homogenised as detailed in Section 3.2.1.

1. Add 250 µL Lysis buffer PN to each homogenised sample. N.B. if fresh tissue has been used, and Lysis buffer PN was used in the homogenisation (Section 3.2.1), no further addition of Lysis buffer PN is required.
  **If RNase A is being used, it should be added during this step (see Section 2.3 for details).**
2. Incubate at 65 °C for >10 min.
3. Centrifuge at 2500 x g for 10 min 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. Please 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 min to allow binding to occur.
7. Bring magnet into contact with the tube(s) for 1 min 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 min. Periodically agitate the sample using a shaker or vortexer.
12. Bring magnet into contact with the tube(s) for 1 min 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.
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 min. Periodically agitate the sample using a heated shaker or vortexer. Please 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 min 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.

4. Troubleshooting

Table 2 below outlines common problems that can be experienced when performing DNA extraction using the sbeadex maxi plant kit. The possible causes are detailed, and corrective actions suggested.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR inhibition</td>
<td>Incomplete buffer removal</td>
<td>Ensure all the buffer is removed before adding the next buffer. If necessary adjust the liquid handling parameters for automated systems.</td>
</tr>
<tr>
<td>Low yield</td>
<td>Inefficient binding</td>
<td>Ensure that the lysate, Binding buffer PN and sbeadex particles are mixed thoroughly.</td>
</tr>
<tr>
<td></td>
<td>DNA loss during third wash</td>
<td>The pH of the water used for the third wash must be pH≤7. A higher pH will lead to partial elution of the nucleic acid.</td>
</tr>
<tr>
<td>Coloured eluates</td>
<td>Incomplete buffer removal</td>
<td>Ensure all the buffer is removed before adding the next buffer. If necessary adjust the liquid handling parameters for automated systems.</td>
</tr>
<tr>
<td></td>
<td>Heavily stained sample material</td>
<td>Incomplete lysis. Contact support (<a href="mailto:kits@lgcgroup.com">kits@lgcgroup.com</a>) for advice.</td>
</tr>
<tr>
<td>Particles present in eluates</td>
<td>Aspirating too fast</td>
<td>Reduce the speed (µL/sec) at which supernatants are removed.</td>
</tr>
<tr>
<td></td>
<td>Loose pellet</td>
<td>Increase separation time (i.e. contact time with the magnet) to allow time for a tighter pellet to form.</td>
</tr>
<tr>
<td></td>
<td>Disrupting pellet during aspiration</td>
<td>Position tip further away from pellet whilst removing supernatants.</td>
</tr>
</tbody>
</table>

Table 2. Troubleshooting common issues that may arise during the sbeadex extraction protocol.
5. Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure.
- Binding buffer PN and Wash buffer PN1 contain high concentrations of detergent and salt. **Please note:** In case of accidental contact, thoroughly rinse or flush the affected areas with water.
- Binding buffer PN and Wash buffer PN1 contain up to 50 % n-propanol. Keep away from naked flames.
- Safety data sheets for all buffers included within the sbeadex maxi plant kit are accessible from LGC’s website.

<table>
<thead>
<tr>
<th>Kit component</th>
<th>GHS symbol</th>
<th>Hazard phrases</th>
<th>Precaution phrases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis buffer PN</td>
<td>![Warning]</td>
<td>H319/H400</td>
<td>P264/P273/P280/P305+P351+P338/P337+/P313/P501</td>
</tr>
<tr>
<td>Binding buffer PN</td>
<td>![Danger]</td>
<td>H226/H302/H315/H318/H336</td>
<td>P101/P102/P103/P210/P241/P303+P361+/P353/P305+P351+P338/P310/P501</td>
</tr>
<tr>
<td>sbeadex particle suspension</td>
<td>![Danger]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wash buffer PN1</td>
<td>![Danger]</td>
<td>H226/H315/H318/H336</td>
<td>P210/P303+P361+/P353/P305+P351+P338/P310/P405/P501</td>
</tr>
<tr>
<td>Wash buffer PN2</td>
<td>![Danger]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elution buffer PN</td>
<td>![Danger]</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Overview of safety information for all buffers included within the sbeadex maxi plant kit.

6. Automating the sbeadex extraction protocol

Once the sbeadex DNA extraction protocol has been trialled (and optimised where necessary) for your sample type manually, it is possible to automate the procedure to increase throughput. LGC 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 extractions specialists at LGC (see Section 7). We are able to offer pilot studies and customised protocols where required.

6.1 Mixing of samples

To mix samples efficiently using an automated liquid handling system, LGC recommends the following:

1. Set the mixing volume to between 50 and 80 % of the volume to be mixed (instrument dependent).
2. For each mixing step, aspirate and dispense between 5 and 10 times (dependent on efficiency of the liquid handler).
3. Ensure aspirate and dispense speeds are low for mixing steps involving Lysis buffer PN and Binding buffer PN to prevent frothing.
4. Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete resuspension.
6.2 Automation via Kingfisher
For automation on a Kingfisher system (or similar) we recommend the following:

1. Keep all volumes the same as for manual extraction, 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).
2. The incubation for each bind and wash step should be a minimum of 5 minutes long 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. A standard Kingfisher protocol can be obtained by contacting support (kits@lgcgroup.com)

6.3 Automation via oKtopure
The oKtopure software is supplied with a standard sbeadex maxi plant kit template that can be used for automation of the sbeadex maxi plant kit protocol. When testing the protocol, it is important to observe for the following:

1. Blocked tips due to clumping beads.
2. Effective resuspension of the pellet after addition of wash buffer.
If one of these issues is detected, please contact support for advice (kits@lgcgroup.com).
Please note: A sbeadex maxi plant kit for 960 extractions and optimised for use with the oKtopure can be ordered under product number NAP41640.

7. Further support
If you require any further support with use of the sbeadex maxi plant kit, or would like to discuss options for optimisation, please do not hesitate to contact our team of nucleic acid extraction specialists.

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Email: info.de@lgcgroup.com