



sbeadex mini plant kit

Catalogue numbers **41601** and **41610**

(For research use only. Not for use in diagnostic procedures.)

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1. Introduction

The sbeadex™ mini 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 are 5-10 mg lyophilised tissue or 10-30 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.



Figure 1. sbeadex protocols follow a unique technology which includes a two-step binding mechanism.

2. Kit contents and storage conditions

2.1 sbeadex mini plant kit contents and storage conditions

The kit contents and storage conditions are outlined in Table 1 below (NAP41601 is sufficient for 96 extractions and NAP41610 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.

Component	Label colour	Volume (NAP41601)	Volume (NAP41610)	Storage conditions
Lysis buffer PN	Blue 	15 mL	100 mL	Room temperature
Binding buffer PN	Green 	15 mL	200 mL	Room temperature
sbeadex particle suspension	White 	1.1 mL	11 mL	Room temperature (4 °C after opening)
Wash buffer PN1	Red 	30 mL	200 mL	Room temperature
Wash buffer PN2	Yellow 	30 mL	200 mL	Room temperature (4 °C after opening)
Elution buffer PN	Black 	15 mL	100 mL	Room temperature (4 °C after opening)

Table 1. sbeadex mini 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*

sbeadex 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 sbeadex mini plant kit protocol.

3. Experimental procedure

3.1 Performing the protocol manually

When performing the sbeadex mini 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 sbeadex mini 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 mini 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 90 μ L 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 90 μ L Lysis buffer PN ● to each homogenised sample. Please note: 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 120 μ L Binding buffer PN ● and 10 μ L sbeadex particle suspension ○ to a fresh sample tube. Please note: Ensure that the sbeadex particle suspension ○ is mixed well before use.
5. Transfer 50 μ L 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 μ L 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 200 μ L Wash buffer PN1 ●. Mix thoroughly by pipetting (suggested pipette volume 150 μ L) 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 200 μ L Wash buffer PN2 ●.
16. Repeat steps 10 to 14 with 200 μ L sterile ultrapure water.
17. Add 70 μ L Elution buffer PN ● to the pellet. Mix thoroughly by pipetting (suggested pipette volume 65 μ L), 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 50 μ L of the eluate.

4. Troubleshooting

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

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

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 mini plant kit are accessible from LGC's website.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer PN 	 Warning	H319/H400	P264/P273/P280/P305+P351+P338/P337+P313/P501
Binding buffer PN 	 Danger	H226/H302/H315/ H318/H336	P210/P241/P303+P361+P353/P305+P351+P338/P310/ P501
sbeadex particle suspension 	-	-	-
Wash buffer PN1 	 Danger	H226/H315/H318/ H336	P210/P303+P361+P353/P305+P351+P338/P310/P405/ P501
Wash buffer PN2 	-	-	-
Elution buffer PN 	-	-	-

Table 3. Overview of safety information for all buffers included within the sbeadex mini 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 at kits@lgcgroup.com.

6.3 Automation via oKtopure

The oKtopure software is supplied with a standard sbeadex mini plant kit template that can be used for automation of the sbeadex mini 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 at kits@lgcgroup.com.

Please note: A sbeadex mini plant kit for 960 extractions and optimised for use with the oKtopure can be ordered under product number NAP41607.

7. Further support

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

Telephone: +49 (0)30 5304 2200

Email: info.de@lgcgroup.com

www.lgcgroup.com/genomics • genomics@lgcgroup.com

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