Application note SPAK-0006

sbeadex[®] mini plant kit and KingFisher 96 instrument

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Description

The sbeadex[®] mini plant kit (Cat. No. 41601 and 41610) has been developed to extract genomic DNA from a wide variety of plant materials (leaves, seeds, fruits, etc). The magnetic particle based DNA extraction protocol can be easily automated using a KingFisher 96 (Thermo Fisher Scientific) magnetic particle manipulator. Using magnetic rods, which are protected against contamination by a tip comb, the magnetic beads are transferred from one buffer plate to the next during the extraction process. This instrument can process up to 96 samples per run. In addition, the KingFisher 96 instrument is compatible with liquid handling systems and sample handling devices, thus making a hands-free medium to high throughput system a reality.

sbeadex[®] coated magnetic particles bind DNA using a novel two-step binding mechanism in the presence of detergents and salts. After binding and washing steps, the purified DNA is released in the elution buffer. The sbeadex[®] mini plant kit is supplied with ready-to-use buffers. Kits are also available for large scale (sbeadex[®] maxi plant kit) extraction. The processing time on the KingFisher 96 is approximately 30 minutes.

The method described here is a universal plant protocol which can be used for a wide range of plant types without adaptation. Whenever necessary, customisation of the protocol is possible using the software provided with the instrument.

- A copy of the instrument protocol is available on request (email: extraction@lgcgenomics.com)
- The instrument protocol is compatible with the KingFisher software version 2.6.22
- For tips and advice on how to adapt the instrument protocol for the BindIt[™] software of the KingFisher Flex instrument, please email extraction@lgcgenomics.com
- See the sbeadex[®] mini plant kit protocol for further information about the kit, limitations of product use, safety information etc.

Equipment and reagents

Product description	Cat. No.	Labware required per run
sbeadex [®] mini plant kit (96 tests)	41601	-
sbeadex [®] mini plant kit (960 tests)	41610	-
KingFisher 96 magnetic particle processor	5400500*	-
KingFisher 96 DW magnet	24073430*	-
KingFisher 96 tip comb for DW magnet	97002534*	1
KingFisher 96 KF plate 200 µL	97002540*	5
Ultrapure water (not part of the kit)	-	-

 Table 1: Equipment and reagents required for DNA extraction using sbeadex[®] mini plant kits on KingFisher 96.

 * supplied by Thermo Fisher Scientific







Importing instrument protocol

To save the instrument protocol to your computer:

- 1. Open KingFisher software
- 2. Select Cancel in the Startup window
- 3. Select Protocol → Import/Export data
- Click Read file on the left side of the 'Import/Export protocols' window. An 'Open' window appears
- Select the protocol you want to import ('sbx_miniplant_KF96.kf2') and click Open
- 6. The protocol appears in the 'Protocols in file' list
- Select protocol 'sbx_miniplant_KF96.kf2' in the 'Protocols in file' list and click Import
- 8. A message will appear that the update of the database was successful
- Now you can start the protocol directly from the software or transfer it to the KingFisher 96 instrument
- 10. Select Instrument \rightarrow Send protocol to Instrument
- 11. Select the protocol ('sbx_miniplant_KF96.kf2') from the list 'Protocols for selected instrument' and click Send protocol
- After the transfer of the protocol to the KingFisher
 96 instrument a message will appear indicating the successful transfer.

Instrument procedure sbeadex® mini plant kit

- 1. Fill the following deep well/ KingFisher plates with sbeadex mini plant kit reagents as specified in table 2:
 - Add 200 µL of the tissue samples into wells of a deep well plate 'Binding_Pos1'
 - Plate 'Wash1_Pos2'
 - Plate 'Wash2_Pos3'
 - Plate 'Wash3 Pos4'
 - Plate 'Elution_Pos5'
- 2. The protocol **sbx_miniplant_KF96** is designed to extract genomic DNA from plant material. Up to 20 -30 mg of plant material can be used for this protocol
- 3. Add 90 µL of Lysis buffer PN to the sample material and grind thoroughly, e.g. in a ball mill. For some materials, such as monocotyledonous plant leaves, grinding in dry ice (without the lysis buffer) or freezing in liquid nitrogen first will improve the homogenisation efficiency
- 4. Incubate samples at 65 °C for at least 10 min. Then centrifuge the mixture for 10 min at maximum speed
- 5.Remove 50 µL supernatant and transfer it to the prepared plate 'Binding_Pos1' (see Table 2)
- 6.Select the **sbx_miniplant_KF96** protocol on the KingFisher 96 instrument
- Load the prepared plates as prompted by the software and start the instrument
- After approximately 30 min the protocol will be finished and the plant DNA is ready for downstream analysis.

Plate name in protocol	Plate type	Well content	Volume
Comb_Pos6	KingFisher 96 KF plate	Tip comb	-
Binding_Pos1 KingFisher 96 KF plate	Lysate	50 µL	
		Binding buffer PN	120 µL
		Particle suspension PN	10 µL
Wash1_Pos2	KingFisher 96 KF plate	Wash buffer PN 1	200 µL
Wash2_Pos3	KingFisher 96 KF plate	Wash buffer PN 2	200 µL
Wash3_Pos4	KingFisher 96 KF plate	Ultrapure water	200 µL
Elution_Pos5	KingFisher 96 KF plate	Elution buffer PN	70 µL

Table 2: Plate filling instructions for KingFisher 96 and sbx_miniplant_KF96 protocol.



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