sbeadex Plasmid DNA Purification Kit and KingFisher 96 instrument

Protocol

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

1. Description

The sbeadex™ Plasmid DNA Purification Kit has been developed for high throughput extraction of plasmid DNA from bacterial cultures. 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 Plasmid DNA Purification Kit is supplied with ready-to- use buffers. The processing time with KingFisher 96 is approximately 20 minutes. Typically, plasmid DNA extraction from a 1 mL bacterial culture with KingFisher 96 and sbeadex Plasmid DNA Purification Kit results in about 5-10 µg of high-quality plasmid DNA.

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

Notes

- A copy of the instrument protocol is available on request (email: techsupport@lgcgroup.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 BindItTM software of the KingFisher Flex instrument please email techsupport@lgcgroup.com
- See the sbeadex Plasmid DNA Purification Kit manual for further information about the kit, limitations of product use, safety information etc.

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2. Equipment and reagents

Product description	Cat. no.	Labware required per run
sbeadex Plasmid DNA Purification Kit (96 tests)	NAP41301	-
sbeadex Plasmid DNA Purification Kit (960 tests)	NAP41310	-
KingFisher 96 magnetic particle processor	Available from Thermo Fisher Scientific	-
KingFisher 96 DW magnet		-
KingFisher 96 tip comb for DW magnet	NAP40000D*	1
KingFisher 96 plate 200 µL	NAP40000H*	3
DW 96 plate,V-bottom, polypropylene	NAP40000F*	2
Ultrapure water (not part of the kit)	User supplied	-
2 - propanol (not part of the kit)	User supplied	-

Table 1. Equipment and reagents required for DNA extraction using sbeadex Plasmid DNA Purification Kit on KingFisher 96.
* supplied by Thermo Fisher Scientific

3. Importing KingFisher 96 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**.
4. Click **Read file** on the left side of the 'Import/Export protocols' window. An 'Open' window appears.
5. Select the protocol you want to import **('sbx_plasmid_KF96.kf2')** and click **Open**.
6. The protocol appears in the 'Protocols in file' list.
7. Select protocol **'sbx_plasmid_KF96.kf2'** in the 'Protocols in file' list and click **Import**.
8. A message will appear that the update of the database was successful.
9. Now you can start the protocol directly from the software or transfer it to the KingFisher 96 instrument.
10. Select **Instrument** → **Send protocol to Instrument**.
11. Select the protocol **('sbx_plasmid_KF96.kf2')** from the list 'Protocols for selected instrument' and click **Send protoco**l.
12. After the transfer of the protocol to the KingFisher 96 instrument a message will appear indicating the successful transfer.

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4. Instrument procedure Thermo Fisher

1. Prepare Resuspension buffer PP 1 working solution by adding the required amount of RNase A according to the instructions given in the sbeadex Plasmid DNA Purification Kit manual.
2. Fill the following deep well/ KingFisher plates with sbeadex Plasmid DNA Purification Kit reagents as specified in table 2.

- Plate ‘Binding_Pos1’: (2-Propanol and sbeadex particle suspension PLN only. Ensure the magnetic particles are thoroughly re-suspended before dispensing.)
- Plate ‘Wash1_Pos2’
- Plate ‘Wash2_Pos3’
- Plate ‘Wash3_Pos4’
- Plate ‘Elution_Pos5’

3. The protocol **sbx_plasmid_KF96** is designed to extract plasmid DNA from bacterial cultures.
4. Harvest bacterial cells by centrifuging (e.g. in a DW 96 plate, V-bottom) for 5 mins at 2000 g and discard the supernatant.
5. Resuspend pellet in 130 µL of precooled Resuspension buffer PP 1 working solution by vortexing.
6. Add 130 µL of Lysis buffer PP 2 mix gently and then incubate for 7 min at room temperature.
7. Add 130 µL of Neutralisation buffer PP 3 and mix gently.
8. To remove cell debris and chromosomal DNA centrifuge for 15 min at maximum speed.
9. Transfer 200 µL of supernatant into the prepared ‘Binding_Pos1’ plate and tip mix three times.
10. Select the **sbx_plasmid_KF96** protocol on the KingFisher 96 instrument.
11. Load the prepared plates as prompted by the software and start the instrument.
12. After approximately 20 min the protocol will be finished, and the plasmid DNA is ready for downstream analysis.

Plate name in protocol	Plate type	Well content	Volume
Comb_Pos5	KingFisher 96 KF plate	Tip comb	-
Binding_Pos1	DW 96 plate, V-bottom	Cleared lysate	200 µL
		2-Propanol	200 µL
		sbeadex particle suspension	10 µL
Wash1_Pos2	DW 96 plate, V-bottom	Wash buffer PN 2	500 µL
Wash2_Pos3	DW 96 plate, V-bottom	Ultrapure water	500 µL
Elution_Pos4	KingFisher 96 KF plate	Elution buffer PN	70 µL

Table 2. Plate filling instructions for KingFisher 96 and sbx_plasmid_KF96 protocol.

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