

## sbeadex<sup>®</sup> plasmid kit and KingFisher 96 instrument

F. Schubert, LGC Genomics, Ostendstrasse 25, TGS Haus 8, 12459 Berlin, Germany



### Description

The sbeadex<sup>®</sup> plasmid kit (Cat. No. 41301 or 41310) 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<sup>®</sup> 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<sup>®</sup> plasmid 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<sup>®</sup> plasmid 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: [extraction@lgcgenomics.com](mailto: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<sup>™</sup> software of the KingFisher Flex instrument please email [extraction@lgcgenomics.com](mailto:extraction@lgcgenomics.com)
- See the sbeadex<sup>®</sup> plasmid 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 <sup>®</sup> plasmid kit (96 tests)	41301	-
sbeadex <sup>®</sup> plasmid kit (960 tests)	41310	-
KingFisher 96 magnetic particle processor	5400500*	-
KingFisher 96 DW magnet	24073430*	-
KingFisher 96 tip comb for DW magnet	97002534*	1
KingFisher 96 plate 200 µL	97002540*	3
DW 96 plate, V-bottom, polypropylene	95040450*	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<sup>®</sup> plasmid 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**
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 protocol**
12. After the transfer of the protocol to the KingFisher 96 instrument a message will appear indicating the successful transfer

## Instrument procedure sbeadex® plasmid kit

1. Prepare Re-suspension buffer PP 1 working solution by adding the required amount of RNase A according to the instructions given in the sbeadex® plasmid kit protocol

2. Fill the following deep well/ KingFisher plates with sbeadex® plasmid 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. Re-suspend 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
		Particle suspension PLN	10 µL
Wash1_Pos2	DW 96 plate, V-bottom	Wash buffer PLN 1	500 µL
Wash2_Pos3	DW 96 plate, V-bottom	Ultrapure water	500 µL
Elution_Pos4	KingFisher 96 KF plate	Elution buffer PLN	70 µL

Table 2: Plate filling instructions for KingFisher 96 and sbx\_plasmid\_KF96 protocol.



[www.lgcgenomics.com](http://www.lgcgenomics.com)

### LGC Genomics

#### Germany

Ostendstr. 25 • TGS Haus 8  
12459 Berlin

Tel: +49 (0)30 5304 2200  
Fax: +49 (0)30 5304 2201  
Email: [info.de@lgcgenomics.com](mailto:info.de@lgcgenomics.com)

#### United Kingdom

Unit 1-2 Trident Industrial Estate • Pindar Road  
Hoddesdon • Herts • EN11 0WZ

Tel: +44 (0) 1992 470757  
Fax +44 (0) 1438 900670  
Email: [info.uk@lgcgenomics.com](mailto:info.uk@lgcgenomics.com)

#### USA

100 Cummings Center • Suite 420H  
Beverly • MA 01915

Tel: +1 (978) 232 9430  
Fax: +1 (978) 232 9435  
Email: [info.us@lgcgenomics.com](mailto:info.us@lgcgenomics.com)