Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- Lysis buffer PLN and Neutralisation buffer PLN contain high concentrations of detergent and salt. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Isopropanol is volatile and highly flammable. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Resuspension buffer PLN	-	-	-
Lysis buffer PLN	Danger	H314	P260/P303+P361+P353/ P305+P351+P338/ P310/P405/P501
Neutralisation buffer PLN	Warning	H315/H319	P264/P280/ P305+P351+P338/P332+P313 /P337+P313/P501
RNase A	Danger	H317/H334	P261/P280/P285/P304+P341/ P342+P311
sbeadex particle suspension	-	-	-
Wash buffer PLN	-	-	-
Elution buffer PLN	-	-	-

SDS (Safetydata sheet) are available on our webpage www.lgcgroup.com/genomics.

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sbeadex plasmid kit

Catalogue number **41301** and **41310**
(For research use only. Not for use in diagnostic procedures.)

Description

sbeadex™ kits use magnetic separation for the preparation of nucleic acids. Superparamagnetic particles coated with sbeadex surface chemistry are used to capture nucleic acids from a sample. sbeadex utilises a novel two step binding mechanism which combined with the washing steps removes impurities present in the sample matrix. After washing the nucleic acid is eluted and is ready for use in downstream processes.

Kit uses

sbeadex plasmid kits are used to extract plasmid DNA from bacterial cultures for use in sequencing and PCR related technologies. The method was developed and optimised using 1 mL of bacterial culture.

For information on protocols for other starting materials please contact our application specialists via email: info.de@lgcgroup.com or Tel: +49 (0)30 5304 2200.

Kit content

	Colour	Cat. 41301	Cat. 41310
Resuspension buffer PLN	Blue	15 mL	200 mL
Lysis buffer PLN	Blue	15 mL	200 mL
Neutralisation buffer PLN	Green	15 mL	200 mL
RNase A	Grey	14 µL	140 µL
sbeadex particle suspension	White	1,1 mL	11 mL
Wash buffer PLN	Yellow	30 mL	750 mL
Elution buffer PLN	Black	15 mL	100 mL

Additional required reagents:

- Ultra pure sterile water
- Isopropanol

Additional buffers can be purchased separately, catalogue numbers available on request

Storage

Kit components should be used within twelve months of delivery and stored under the recommended conditions. Please refer to the kit box label for the expiry date.

Room temperature	4 °C
Resuspension buffer PLN (without RNase A) Lysis buffer PLN Neutralisation buffer PLN sbeadex particle suspension Wash buffer PLN Elution buffer PLN	RNase A (Note: Once RNase A has been added to Resuspension buffer PLN the buffer should be kept at 4°C)

Troubleshooting

Problem	Possible cause	Corrective action
PCR inhibition	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer. Check and if necessary adjust the liquid handling parameters for automated systems
Low yield	Inefficient binding	Ensure that the clear lysate, isopropanol and sbeadex particles are mixed thoroughly
	DNA loss during 3rd wash	The pH of the water used for the 3rd wash must be </= 7. Higher pH will lead to partial elution of the nucleic acid
Particles present in eluates	Aspirating too fast	Reduce the speed at which supernatants are removed
	Loose pellet	Increase separation time to allow time for a tighter pellet to form
	Disrupting pellet during aspiration	Position tip further away from pellet whilst removing supernatants

Tips for automated protocol

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. Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete resuspension.

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, 20µL additional Elution buffer should be added to the elution plate.
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 ^{o}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.
4. A standard Kingfisher protocol can be obtained by contacting support (kits@lgcgroup.com)

Elution at room temperature

- Elution can be carried out at room temperature if necessary. However be aware that a ~20 % drop in DNA yield is seen in samples with high concentrations of DNA e.g. buccal swabs when elution is performed at room temperature
- For those wanting as high a yield as possible elution at 55 ℃ is recommended.

Reagent preparation

Presence of precipitates

Salt precipitates can form in Lysis buffer PLN and Neutralisation buffer PLN at low temperatures. Check for the presence of precipitates prior to use and if required re-dissolve them by incubating the buffers at 37°C.

sbeadex particle suspension

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

Resuspension buffer PLN with RNase A

Prepare the Resuspension buffer PLN/RNase A mix by adding 1 µL of RNase A to every 1 mL of Resuspension buffer PLN. Mix thoroughly. When not in use store the Resuspension buffer PLN/RNase A mix at 4°C. The Resuspension buffer PLN/RNase A mix is stable for six months.

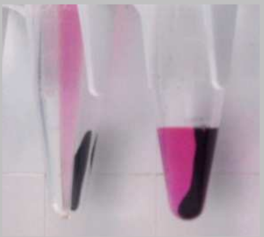
Manual protocol

1. Pre-cool **Resuspension buffer PLN**
2. Harvest bacterial cells by centrifuging samples in a deep well plate at 2 000 g for 10 minutes then remove the supernatant
3. Add 130 µL of **Resuspension buffer PLN/ RNase A** to the pellet and vortex to re-suspend the pellet
4. Add 130 µL of **Lysis buffer PLN** to each sample and vortex for 5 seconds
5. Incubate at room temperature for 7 minutes
6. Add 130 µL of **Neutralisation buffer PLN** to each sample and vortex for 5 seconds
7. Centrifuge at 2 000 g for 15 minutes
8. Add 200 µL of **isopropanol** to a fresh sample tube
9. Ensure the **sbeadex® particles** are fully re-suspended. Add 10 µL to the tubes containing the isopropanol
10. Transfer 200 µL of the **clear lysate** to the tubes containing the isopropanol/ sbeadex® particles. Mix thoroughly, set pipette volume to 350 µL and pipette up and down 5 times
11. Incubate for 4 minutes at room temperature to allow sufficient time for binding to occur
12. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex® particles to form a pellet
13. Remove the supernatant and discard. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet
14. Move the magnet away from the sample tubes
15. Add 500 µL of **Wash buffer PLN** and re-suspend the pellet. Mix thoroughly, set pipette volume to 450 µL and pipette up and down 5 times or until pellet is fully re-suspended
16. Incubate at room temperature for 10 minutes, agitating the sample during the time period. Use a shaker or vortex periodically
17. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet
18. Remove the supernatant and discard. Ensure as much of the supernatant is removed as is possible without dislodging the particle pellet
19. Move the magnet away from the sample tubes
20. Repeat steps 15 to 19 with 500 µL of **ultra pure water**
21. Add 63 µL of **Elution buffer PLN** and re-suspend the pellet. Mix thoroughly, set pipette volume to 50 µL and pipette up and down 5 times or until pellet is fully re-suspended

22. Incubate at 55°C for 10 minutes, agitating the sample during the time period. Use a heated shaker or vortex periodically
23. Bring magnet into contact with the sample tubes. Wait for 3 minutes at room temperature to allow the sbeadex particles to form a pellet
24. Remove the eluate and place into a new sample tube. To avoid particle transfer it is recommended to transfer only 50 µL of the eluate.

Tips for manual protocol

For manual testing of the protocol or if no magnet is available it is recommended to spin tubes for 10 seconds to enable the magnetic particles to form a pellet.



When removing supernatants it is important to remove as much of the liquid as possible without dislodging the particle pellet. With magnets used for manual protocols the particle pellet forms on the back wall of the sample tube. When placing the pipette tip inside the tube be sure to aim the end of the tip to the front wall of the sample tube to avoid disrupting the particle pellet.

To remove as much liquid as possible it is recommended to aspirate once, let any liquid run down the walls of the tube and then aspirate a second time to remove these remnants of liquid.