Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- Lysis buffer TN, Binding buffer TN and Wash buffer TN 1 contain high concentrations of detergent and salt. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Wash buffer TN 1 contains up to 50 % n-propanol. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer TN	-	-	-
Protease	 Danger	H315/H319/H334/ H335	P261/P305+P351+P338/ P342+P311
Binding buffer TN	 Warning	H302/H315/H319	P280/P305+P351+P338/P310/ P362/ P332+P313/P501
Binding additive TN	 Danger	H315/H318/H330	P260/P280/P284/ P305+P351+P338/P310
sbeadex particle suspension	-	-	-
Wash buffer TN 1	 Danger	H225/H315/H318/ H336	P210/P303+P361+P353/P305+ P351+P338/P310/P405/P501
Wash buffer TN 2	-	-	-
Elution buffer TN	-	-	-

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

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sbeadex tissue kit

Catalogue number **41405** and **41450**
(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 tissue kits are used to extract DNA from animal tissue. The method was developed and optimised using 20 mg of animal tissue. The following tissue types have been tested and found to be compatible with sbeadex nucleic acid extraction kits:

- Mouse tails
- Mouse lung
- Ear punches
- Skin
- Spleen
- Muscle

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

Kit content

	Colour	Cat. **41405**	Cat. **41450**
Lysis buffer TN	Blue	15 mL	60 mL
Protease	Grey	32 mg	320 mg
Binding buffer TN	Green	15 mL	60 mL
Binding additive TN	-	1 mL	9 mL
sbeadex particle suspension	White	1,5 mL	15 mL
Wash buffer TN 1	Red	30 mL	200 mL
Wash buffer TN 2	Yellow	30 mL	200 mL
Elution buffer TN	Black	15 mL	100 mL

Additional required reagents:

- Ultra pure sterile water
- n-propanol

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	-20 °C
Lysis buffer TN Binding buffer TN Binding additive TN sbeadex particle suspension Wash buffer TN 1 Wash buffer TN 2 Elution buffer TN	Protease

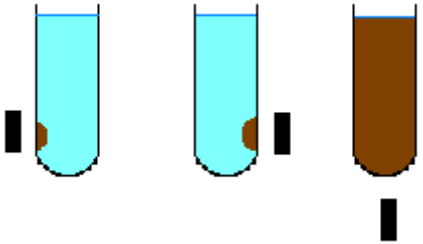
Troubleshooting

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	Poor protease activity	Prepare the protease as detailed in the 'Reagent preparation' section, aliquot into several tubes and store -20 °C. Remove and thaw aliquots as required. Do not use protease which has been kept at room temperature for an extended period of time
	Inefficient binding	Ensure that the lysate, n-propanol 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
Coloured eluates	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
	Heavily stained sample material	Contact our technical specialists for advice
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

Using sep™ boxes (cont'd.)

- The magnets can be placed in three positions in relation to the sample – left, right and underneath (away from the sample)



- For effective re-suspension of particle pellets it is recommended to move the magnets from the left to right positions using the 'cycle mode'. See sep box operating manual for more details
- For efficient elution of the nucleic acids from the particles it is recommended to use the 'cycle mode' during the elution incubation period.

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 when elution is performed at room temperature
- For those wanting as high a yield as possible elution at 55 °C is recommended.

Reagent preparation

Presence of precipitates

Salt precipitates can form in Lysis buffer TN and Binding Buffer TN 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.

Protease

Prepare the Protease by adding the appropriate amount (see table below) of pure water to the vial of Protease. When not in use store the Protease at -20 °C.

Kit catalogue number	Volume of pure water
41405	1.6 mL
41450	16 mL

Binding mix

To reduce the number of pipetting steps a binding mix can be prepared at the end of the lysis incubation period. Add 8 µL of Binding additive TN to 42 µL of Binding buffer TN for the number of samples to be processed. The table below gives some example calculations including a 10 % wastage factor. Mix thoroughly. Use the binding mix within 30 minutes.

No. of samples	Vol. of Binding additive TN	Vol. of Binding buffer TN
1	8.8 µL	46.2 µL
5	44 µL	231 µL
20	176 µL	924 µL
96	844.8 µL	4.4 mL

sbeadex particle suspension

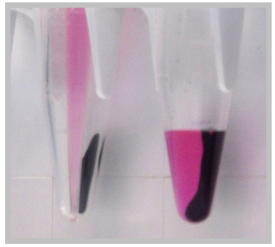
Mix the suspension thoroughly before use to fully re-suspend the particles.

Manual protocol

1. Add 60 µL of **Lysis buffer TN** and 15 µL of **Protease** to 20 mg of sample material. Mix thoroughly by vortexing
2. Incubate at 55 °C overnight or until the tissue is completely dissolved
3. To a fresh sample tube add 42 µL of **Binding buffer TN** and 8 µL of **Binding additive TN** and 60 µL of **n-propanol**
4. Ensure the **sbeadex particles** are fully re-suspended. Add 15 µL to the tubes containing the binding mix
5. Transfer 50 µL of the **sample lysate** to the tube containing the binding mix and mix thoroughly, set pipette volume to 150 µL and pipette up and down 5 times
6. Incubate for 3 minutes at room temperature to allow sufficient time for binding to occur
7. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the sbeadex particles to form a pellet
8. Remove the supernatant and discard. Ensure as much of the supernatant is removed as possible without dislodging the particle pellet
9. Move the magnet away from the sample tubes
10. Add 200 µL of **Wash buffer TN 1** and re-suspend the pellet. Mix thoroughly, set pipette volume to 150 µL and pipette up and down 5 times or until pellet is fully re-suspended
11. Incubate at room temperature for 10 minutes, agitating the sample during the time period. Use a shaker or vortex periodically
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 possible without dislodging the particle pellet
14. Move the magnet away from the sample tubes
15. Repeat steps 10 to 14 with 200 µL of **Wash buffer TN 2**
16. Repeat steps 10 to 14 with 200 µL of **pure water**
17. Add 63 µL of **Elution buffer TN** 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
18. Incubate at 55 °C for 10 minutes, agitating the sample during the time period. Use a heated shaker or vortex periodically
19. Bring magnet into contact with the sample tubes. Wait for 3 minutes at room temperature to allow the sbeadex® particles to form a pellet
20. 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.

Tips for automated protocol

Follow the manual protocol as specified overleaf in respect to volumes. Tips on automated mixing are given below:

Mixing with automated liquid handling system

- Set mixing volume to be between 50 % to 80 % of the volume to be mixed (instrument dependent)
- For each mixing step aspirate and dispense between 5 and 10 times depending on the efficiency of the liquid handler
- Keep mix aspirate and dispense speeds low with Lysis buffer TN and Binding buffer TN to avoid frothing
- Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete re-suspension.

Using sep™ boxes

- sep boxes are computer driven magnetic particle collectors with active cooling and heating functionality
- Depending on the sep box used the volumes specified in the manual protocol may need to be changed to be within their maximum working volume. Note: sep 96 x *0.2* has a maximum working volume of 180 µL.