# Safety information



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

| Kit component | GHS symbol | Hazard phrases | Precaution phrases |
|---|---|---|---|
| Lysis buffer FN | - | - | - |
| Protease |  Danger | H315/H319/H334/ H335 | P261/P305+P351+P338/ P342+P311 |
| Binding buffer FN |  Danger | H225/H302/H315/ H318/H336 | P210/P303+P361+P353/P305 +P351+P338/P310/P405/P501 |
| sbeadex particle suspension | - | - | - |
| Wash buffer FN 1 |  Danger | H225/H315/H318/ H336 | P210/P303+P361+P353/P305 +P351+P338/P310/P405/P501 |
| Wash buffer FN 2 | - | - | - |
| Elution buffer FN | - | - | - |

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

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41510/4.00-2016-06



# sbeadex forensic kit

Catalogue number **41501** and **41510**
*(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 forensic kits are used to extract DNA from forensic material. The method was developed and optimised using the following sample types:

- Buccal swabs
- Dried blood spots
- Saliva stains e.g. on cigarettes, drink containers, chewing gum
- Cellular material e.g. on worn clothing, tools, jewellery
- Hair roots
- Fingernails
- Semen

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. **41501** | Cat. **41510** |
|---|---|---|---|
| Lysis buffer FN | Blue | 15 mL | 200 mL |
| Protease | Grey | 4,8 mg | 50,4 mg |
| Binding buffer FN | Green | 30 mL | 200 mL |
| sbeadex particle suspension | White | 1,1 mL | 11 mL |
| Wash buffer FN 1 | Red | 30 mL | 300 mL |
| Wash buffer FN 2 | Yellow | 30 mL | 300 mL |
| Elution buffer FN | Black | 15 mL | 100 mL |

**Additional required reagents:**

- Ultra pure sterile water

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 FN<br>Binding buffer FN<br>sbeadex particle suspension<br>Wash buffer FN 1<br>Wash buffer FN 2<br>Elution buffer FN | Protease |

# Troubleshooting



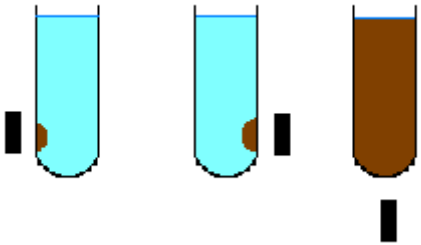
| Problem | Possible cause | Corrective action |
|---|---|---|
| **PCR inhibition** | Incomplete buffer removal | Ensure all the buffer is removed before adding the next buffer. Check and if necessary adjust the liquid handling parameters for automated systems |
| **Low yield** | Poor protease activity | 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, Binding buffer FN 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

- 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.
- 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 e.g. buccal swabs 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 FN at low temperatures. Check for the presence of precipitates prior to use and if required re-dissolve them by incubating the buffer at 37 $^{o}$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 $^{o}$C.

| Kit catalogue number | Volume of pure water |
|---|---|
| 41501 | 600 µL |
| 41510 | 6.3 mL |

### Lysis mix

To reduce the number of pipetting steps a lysis mix can be prepared at the start of the process. Thaw the Protease thoroughly. Add 5 µL of Protease to 115 µL of Lysis buffer FN for the number of samples to be processed. The table below gives some example calculations including a 10 % wastage factor. Mix thoroughly.

| Number of samples | Vol. of Lysis buffer FN | Vol. of Protease |
|---|---|---|
| 1 | 126.5 µL | 5.5 µL |
| 5 | 632.5 µL | 27.5 µL |
| 20 | 2.5 mL | 110 µL |
| 96 | 12.1 mL | 528 µL |

### sbeadex particle suspension

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

# Manual protocol

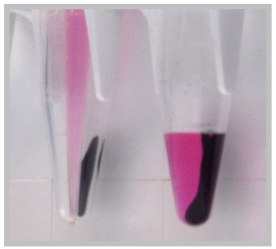


1. Add 115 µL of **Lysis buffer FN** and 5 µL of **Protease** to each sample. Vortex for 5 seconds
2. Incubate at 55 $^{o}$C for 10 minutes
3. Add 200 µL of **Binding buffer FN** to a fresh sample tube
4. Ensure the **sbeadex particles** are fully re-suspended. Add 10 µL to the tubes containing the binding buffer
5. Transfer 100 µL of **lysate** to the tubes containing the binding buffer/sbeadex particles. Mix thoroughly, set pipette volume to 250 µL and pipette up and down 5 times
6. Incubate for 2 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 is possible without dislodging the particle pellet
9. Move the magnet away from the sample tubes
10. Add 300 µL of **Wash buffer FN 1** and re-suspend the pellet. Mix thoroughly, set pipette volume to 250 µL and pipette up and down 5 times or until pellet is fully re-suspended
11. Incubate at room temperature for 5 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 is possible without dislodging the particle pellet
14. Move the magnet away from the sample tubes
15. Repeat steps 10 to 14 with 300 µL of **Wash buffer FN 2**
16. Repeat steps 10 to 14 with 300 µL of **pure water**
17. Add 100 µL of **Elution buffer FN** and re-suspend the pellet. Mix thoroughly, set pipette volume to 75 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 85 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.

One way to remove the lysate from a swab is to perform ‘back pack’ centrifugation. For more details on how this can be carried out in a single tube or plate format contact our application specialists via email: extraction@lgcgenomics.com or Tel: +49 (0)30 5304 2250.

# 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 FN and Binding buffer FN to avoid frothing
- Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete re-suspension.