- Wear appropriate skin and eye protection throughout the extraction procedure
- Binding buffer BL and mag particle suspension BL contain high concentrations of salts and detergents. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Note Binding buffer BL can yellow over time, this change of colour does not affect the performance of the buffer
- Prepared Wash buffer BL 1 and Wash buffer BL 2 contain up to 70 % organic solvents. Keep away from naked flames

| Kit component                  | GHS<br>symbol | Hazard phrases               | Precaution phrases                                    |
|--------------------------------|---------------|------------------------------|---|
| Lysis buffer BL                | -             | -                            | -   |
| Protease                       | Panger Danger | H315/H319/H334/<br>H335      | P261/P305+P351+P338/ P342+P311                        |
| Binding buffer BL              | Danger        | H302+H312+H332/<br>H314/H412 | P260/P280/P305+P351+P338/<br>P312/P405/P501           |
| mag particle-<br>suspension BL | Danger        | H314                         | P260/P303+P361+P353/P305+P351+<br>P338/P310/P405/P501 |
| Wash buffer BL 1 (concentrate) | -             | -                            | -   |
| Wash buffer BL 2 (concentrate) | -             | -                            | -   |
| Elution buffer BL              | -             | -                            | -   |

**SDS** (Safety data sheet) are available at our "Genomics Resource Center" on our webpage www.lgcgroup.com/genomics.



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# mag mini kit

Catalogue number 40401 and 40410

(For research use only. Not for use in diagnostic procedures.)

# **Description**

mag<sup>™</sup> kits use magnetic separation for the preparation of nucleic acids. Superparamagnetic particles coated with mag surface chemistry are used to capture nucleic acids from a sample. The nucleic acid/particle complex is subsequently washed to remove impurities. The nucleic acid is then eluted from the particles and ready for use in downstream processes.



### Kit uses

mag mini kits are used to extract DNA from whole blood and blood products. The method was developed and optimised using 10  $\mu$ L of whole blood. The following anticoagulants have been tested and found to be compatible with mag nucleic acid extraction kits:

- FDTA
- Heparin
- Citrate

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.

|                                | Colour | Cat. <b>40401</b> | Cat. <b>40410</b> |
|--------------------------------|--------|-------------------|-------------------|
| Lysis buffer BL                | Blue   | 15 mL             | 45 mL             |
| Protease                       | Grey   | 4.8 mg            | 50.4 mg           |
| Binding buffer BL              | Green  | 15 mL             | 200 mL            |
| mag particle suspension BL     | White  | 1.2 mL            | 11 mL             |
| Wash buffer BL 1 (concentrate) | Red    | 8,6 mL            | 57 mL             |
| Wash buffer BL 2 (concentrate) | Yellow | 9 mL              | 30 mL             |
| Elution buffer BL              | Black  | 15 mL             | 100 mL            |

#### Additional required reagents:

- Ultra pure sterile water
- Ethanol
- Acetone
- 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.

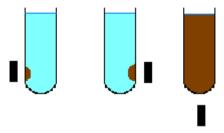
| Room temperature           | -20 °C   |
|----------------------------|----------|
| Lysis buffer BL            |          |
| Binding buffer BL          |          |
| mag particle suspension BL | Protease |
| Wash buffer BL 1           |          |
| Wash buffer BL 2           |          |
| Elution buffer BL          |          |

| Problem                               | Possible cause                  | Corrective action   |  |
|---------------------------------------|---------------------------------|---|--|
| PCR inhibition                        | Incomplete buffer               | Ensure all the buffer is removed before                             |  |
|                                       | removal                         | adding the next buffer. Check and if                                |  |
|                                       |                                 | necessary adjust the liquid handling                                |  |
|                                       |                                 | parameters for automated systems                                    |  |
| Low yield                             | Poor protease                   | Prepare the protease as detailed in the                             |  |
|                                       | activity                        | '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 BL and                       |  |
|                                       |                                 | mag particles are mixed thoroughly                                  |  |
| Wash buffer BL 2                      |                                 | Ensure that the Wash buffer BL 2 bottle is                          |  |
|                                       | acetone                         | closed tightly when not in use to prevent                           |  |
|                                       | composition <70 %               | acetone evaporation   |  |
| Coloured eluates                      | Incomplete buffer               | Ensure all the buffer is removed before                             |  |
|                                       | removal                         | 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               | Position tip further away from pellet whilst                        |  |
|                                       | during aspiration               | removing supernatants   |  |
| Low ratio between                     | Acetone carryover               | Acetone has a maximum UV absorbance at                              |  |
| A <sub>260</sub> and A <sub>280</sub> | in eluate                       | 268 nm and a A <sub>260</sub> /A <sub>280</sub> of 1.53. If this    |  |
|                                       |                                 | phenomenon occurs prolong the drying time                           |  |
|                                       |                                 | to ensure all the acetone evaporates                                |  |

## Reagent preparation

#### 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.

#### Presence of precipitates

Salt precipitates can form in Lysis buffer BL, Binding buffer BL and mag particle suspension BL at low temperatures. Check for the presence of precipitates prior to use and if required redissolve them by incubating the reagents at 37 °C.

#### **Protease**

Prepare the Protease by adding the appropriate amount of pure water to the vial of Protease. For kit catalogue number 40401 add 600  $\mu$ L. For kit catalogue number 40410 add 6.3 mL. When not in use store the Protease at -20 °C.

#### 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  $\mu$ L of Protease to 30  $\mu$ L of Lysis buffer BL for the number of samples to be processed. The table below gives some example calculations including a 10 % wastage factor. Mix thoroughly. Use the lysis mix within 30 minutes.

| Number of samples | Vol. of Lysis buffer BL | Vol. of Protease |
|-------------------|-------------------------|------------------|
| 1                 | 33 µL                   | 5.5 µL           |
| 20                | 660 µL                  | 110 µL           |
| 96                | 3.2 mL                  | 528 μL           |

#### mag particle suspension BL

The mag particles are suspended in a specially formulated buffer which avoids rapid sedimentation or clogging of particles during handling. Mix the suspension thoroughly before use to fully re-suspend the particles.

#### Wash buffer BL 1

Prepare the Wash buffer BL 1 according to the instructions on the bottle label. For kit catalogue number 40401 add 7 mL of ethanol and 7 mL of isopropanol. For kit catalogue number 40410 add 63 mL of ethanol and 60 mL of isopropanol. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation.

#### Wash buffer BL 2

Prepare the Wash buffer BL 2 according to the instructions on the bottle label. For kit catalogue number 40401 add 7 mL of acetone. For kit catalogue number 40410 add 70 mL of acetone. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation.

- 1. Ensure blood samples are well mixed prior to starting the protocol
- 2. Add 30  $\mu$ L of Lysis buffer BL and 5  $\mu$ L of Protease to 10  $\mu$ L of blood. Mix thoroughly, set pipette volume to 30  $\mu$ L and pipette up and down 5 times
- 3. Incubate at 55 °C for 10 minutes then allow to cool down to room temperature
- 4. Add 110 µL of Binding buffer BL to each sample
- 5. Ensure the **mag particle suspension BL** is fully re-suspended. Add 10  $\mu$ L to each sample. Mix thoroughly, set pipette volume to 120  $\mu$ L and pipette up and down 5 times
- 6. Incubate for 2 minutes at room temperature to allow sufficient time for binding to occur
- Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag particles to form a pellet
- 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 130  $\mu$ L of **Wash buffer BL 1** and re-suspend the pellet. Mix thoroughly, set pipette volume to 100  $\mu$ L and pipette up and down 5 times or until pellet is fully resuspended
- 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 mag 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. Repeat steps 9 to 13 with 70 µL of Wash buffer BL 2
- 15. Dry the pellet at 55 °C for 5 minutes. Sample tubes must be left open to allow evaporation to occur
- 16. Add 63 μL of **Elution buffer BL** 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
- 17. Incubate at 55 °C for 10 minutes, agitating the sample during the time period. Use a heated shaker or vortex periodically
- 18. Bring magnet into contact with the sample tubes. Wait for 3 minutes at room temperature to allow the mag particles to form a pellet
- 19. 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 BL and Binding buffer BL 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. <u>Note</u>: sep 96 x 0.2 has a maximum working volume of 180 µL.