Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- 5 x Enrichment buffer BLL contains 0.1 % sodium azide which is a harmful chemical. In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Lysis buffer BLM, mag particle suspension BLM and Wash buffer BLM 1 contain high concentrations of salts and detergents. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Prepared Wash buffer BLM 2 contains up to 70 % acetone. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
5 x Enrichment-buffer BLL	Danger	H318/H412	P280/P305+P351+P338/P501
Lysis buffer BLM	Warning	H302/H315/ H319	P280/P270/P305+P351+P338/ P301+P312/P332+P313/P501
Protease	Danger	H315/H319/ H334/H335	P261/P305+P351+P338/ P342+P311
mag particle suspension BLM	Danger	H314	P260/P303+P361+P353/P305+P351+ P338/P310/P405/P501
Wash buffer BLM 1	Danger	H302/H314/H412	P260/P303+P361+P353/ P305+P351+P338/P310/P405/P501
Wash buffer BLM 2 (concentrate)	-	-	-
Elution buffer BLM	-	-	-

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

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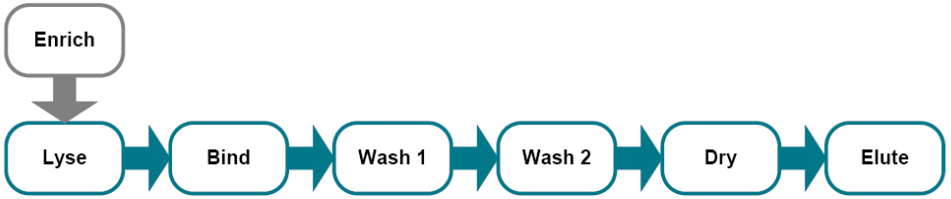
40440/4.00-2016-07

mag maxi PLUS kit

Catalogue number **40404** and **40440**
(For research use only. Not for use in diagnostic procedures.)

Description

mag™ 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 maxi PLUS kits are used to extract DNA from whole blood and blood products. The method was developed and optimised using 2 mL of whole blood with an initial cell enrichment step. The amount of DNA extracted depends on the amount of DNA present in the blood sample (can be affected by the health status of donor). Yields up to 40 µg total DNA are achievable with the mag maxi PLUS kit. The following anticoagulants have been tested and found to be compatible with mag nucleic acid extraction kits:

- EDTA
- 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.

Kit content

	Colour	Cat. **40404**	Cat. **40440**
5 x Enrichment buffer BLL	-	15 mL	375 mL
Lysis buffer BLM	Blue	15 mL	100 mL
Protease	Grey	4,4 mg	156 mg
mag particle suspension BLM	White	220 µL	7,8 mL
Wash buffer BLM 1	Red	15 mL	200 mL
Wash buffer BLM 2 (concentrate)	Yellow	4,5 mL	150 mL
Elution buffer BLM	Black	15 mL	200 mL

Additional required reagents:

- Ultra pure sterile water
- Ethanol
- Acetone

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

Storage

Kit components should be used within 12 months of delivery and stored under the recommended conditions. Please refer to the kit box label for the expiry date.

Room temperature	4 °C	-20 °C
Lysis buffer BLM mag particle suspension BLM Wash buffer BLM 1 Wash buffer BLM 2 Elution buffer BLM	5x Enrichment buffer BLL	Protease

Reagent preparation

Presence of precipitates

Salt precipitates can form in Lysis buffer BLM, mag particle suspension BLM and Wash buffer BLM 1 at low temperatures. Check for the presence of precipitates prior to use and if required re-dissolve them by incubating the reagents at 37 °C.

Different starting blood volumes

With the mag maxi PLUS kit is it possible to extract blood volumes between 1 mL and 3 mL. To adapt the protocol for the different scales of extraction the volume of Enrichment buffer BLL used must be modified. The volume of Enrichment buffer BLL must be twice the volume of blood to be extracted.

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, ethanol and mag particles are mixed thoroughly
	Wash buffer BLM 2 acetone composition <70 %	Ensure that the Wash buffer BLM 2 bottle is closed tightly when not in use to prevent acetone evaporation
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
Low ratio between A_{260} and A_{280}	Acetone carryover in eluate	Acetone has a maximum UV absorbance at 268 nm and a A_{260}/A_{280} of 1.53. If this phenomenon occurs prolong the drying time to ensure all the acetone evaporates

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

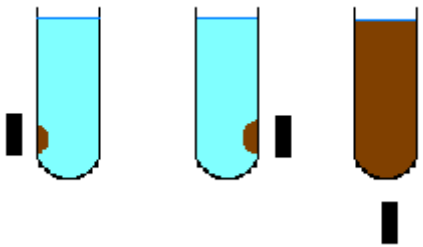
- Set mixing volume to be between 50 % to 80 % of the volume to be mixed (instrument dependent)
- For each mixing step aspirate and dispense between 5 and 10 times depending on the efficiency of the liquid handler

Mixing with automated liquid handling system (cont'd.)

- Keep mix aspirate and dispense speeds low with Lysis buffer BLM 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 72 x *1.4* has a maximum working volume of 1 mL.
- 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.

Reagent preparation

Enrichment buffer BLL

Prepare the Enrichment buffer BLL according to the instructions on the bottle label. For kit catalogue number 40404 add 17 mL of pure water to each bottle and mix well. For kit catalogue number 40440 add 75 mL of pure water to 25 mL of Enrichment buffer BLL concentrate. Mix well. When not in use store Enrichment buffer BLL at 4 °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
40404	220 µL
40440	7.8 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 20 µL of Protease to 200 µL of Lysis buffer BLM 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 BLM	Vol. of Protease
1	220 µL	22 µL
5	1.1 mL	110 µL
20	4.4 mL	440 µL
72	15 mL	1.5 mL

mag particle suspension BLM

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 BLM 2

Prepare the Wash buffer BLM 2 according to the instructions on the bottle label. For kit catalogue number 40404 add 10.5 mL of acetone to the 4.5 mL concentrate. For kit catalogue number 40440 add 350 mL of acetone to the 150 mL concentrate. Mix well. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation.

Manual protocol

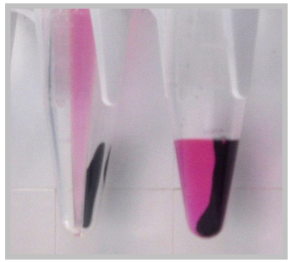
1. Bring **Enrichment buffer BLL** and blood samples to room temperature before starting extraction protocol. Ensure blood samples are well mixed prior to starting the protocol
2. Add 4 mL of **Enrichment buffer BLL** to 2 mL of blood in a 10 mL centrifuge tube. Close the tube tightly and mix well by inverting the tube
3. Incubate at room temperature for 2 minutes, the solution will become clear
4. Centrifuge at 2000 g for 15 minutes. Remove the supernatant carefully without disturbing the pellet
5. Add 200 µL of **Lysis buffer BLM** and 20 µL of **Protease** to a fresh sample tube
6. Re-suspend the pellet in 200 µL of **Enrichment buffer BLL** and transfer it to the tubes containing the Lysis mix. Mix thoroughly, set pipette volume to 350 µL and pipette up and down 5 times
7. Incubate at 55 °C for 10 minutes then allow to cool to room temperature
8. Add 200 µL of **ethanol** to each sample
9. Ensure the **mag particle suspension BLM** is fully re-suspended. Add 20 µL to each sample. Mix thoroughly, set pipette volume to 550 µL and pipette up and down 5 times
10. Incubate for 2 minutes at room temperature to allow sufficient time for binding to occur
11. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag particles to form a pellet
12. Remove the supernatant and discard. Ensure as much of the supernatant is removed as possible without dislodging the particle pellet
13. Move the magnet away from the sample tubes
14. Add 360 µL of **Wash buffer BLM 1** and re-suspend the pellet. Mix thoroughly, set pipette volume to 300 µL and pipette up and down 5 times or until pellet is fully re-suspended
15. Incubate at room temperature for 10 minutes, agitating the sample during the time period. Use a shaker or vortex periodically
16. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag particles to form a pellet
17. Remove the supernatant and discard. Ensure as much of the supernatant is removed as possible without dislodging the particle pellet
18. Repeat steps 13 to 17 with 720 µL of **Wash buffer BLM 2**
19. Repeat steps 13 to 17 a second time with 720 µL of **Wash buffer BLM 2**
20. Dry the pellet at 55 °C for 10 minutes. Sample tubes must be left open to allow evaporation to occur

Manual protocol (cont'd.)

21. Add 200 µL of **Elution buffer BLM** 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
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 mag 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 180 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.