mag maxi kit
bulk kit

For in vitro diagnostic use

CE

IVD

REF **43301** Σ **2500**

May 2014

LGC Genomics GmbH • Ostendstr. 25 • TGS Haus 8 • 12459 Berlin • Germany •
Tel: +49 (0)30 5304 2200 • Fax: +49 (0)30 5304 2201

Intended use of the mag maxi kits

The mag™ maxi kits were developed to isolate human **genomic DNA (gDNA) from whole blood**. This can be done manually according to the protocol in this user manual. The application of the kit on automated liquid handling devices or automated magnetic bead manipulators is possible, as well (see recommendation to automate the kit protocol). mag maxi kits isolate more than 95 % of the gDNA of the sample.
To isolate DNA from other species (e.g. pathogens, bacteria) the chemistry must be reevaluated.
The isolated gDNA can be used as starting material for PCR (Polymerase Chain Reaction) based analysis.

For information on protocols for other starting materials please contact our application specialists via email: extraction@lgcgenomics.com or Tel: +49 (0)30 5304 2250.

Index of contents

Symbols

IVD	In vitro diagnostic medical device	Σ n	Contains sufficient for <n> tests
	Manufacturer		Use by
REF	Catalogue number		Do not reuse
LOT	Batch code		Temperature limitation
	Consult instructions for use		Irritant

Principle of extraction

mag maxi kits use DNA binding magnetic microparticles for the preparation of genomic Deoxyribonucleic Acids (gDNA) from human whole blood.
Superparamagnetic microparticles coated with mag surface chemistry are used to capture gDNA from a lysed blood sample.
The gDNA/particle complex is subsequently washed to remove impurities.
The gDNA is then eluted from the particles and ready for use in PCR based downstream processes.

Kit uses

mag maxi kits are used to extract DNA from whole blood. The method was developed and optimised using 200 µL of fresh or frozen whole human blood.
The following anticoagulants have been tested and found to be compatible with mag maxi extraction kits:

- EDTA
- Citrate

In case you want to extract gDNA from other sample material or blood samples preserved with other anticoagulants, please contact the manufacturer of the kit for consultation and information.

Yield and quality

Average yield of gDNA using the mag maxi kits is between 4-6 µg. UV measurement normally results in a 260/280 ratio (which mirrors contamination with proteins) > 1.75.
Data are given based on manual application of the kit, starting gDNA extraction from healthy patients.
Please note!
gDNA yield depends on many factors. The health conditions of the patient, history of the sample (storage condition, pre-extraction treatment) influence final yield of extraction and are out of the control of the manufacturer of the kit.

Intended user of the mag maxi kits

gDNA extraction must be carried out in appropriate laboratory environment which is designed for working with sample material having human origin.
The user of the kit must have been educated in general treatment of sample material of human origin.
Always wear appropriate protection gloves, a lab coat and suitable eye protection during the work with the mag maxi kits.

Kit content

	Colour	Cat. **43301**
Lysis buffer BLM	Blue	550 mL
Protease	Grey	1120 mg
mag particle suspension BLM	White	58 mL
Wash buffer BLM 1	Red	2000 mL
Wash buffer BLM 2 (concentrate)	Yellow	1200 mL
Elution buffer BLM	Black	550 mL
Handbook		1

Additional required reagents:

- Ultra pure sterile water
- Ethanol (96-100 %)
- Acetone

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

Storage

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

Room temperature (20 – 25 °C)	**-20 °C**
Lysis buffer BLM mag particle suspension BLM Wash buffer BLM 1 Wash buffer BLM 2 Elution buffer BLM	Protease

Safety information

- Wear appropriate skin and eye protection during the usage of the mag maxi kits
- 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 70 % acetone or ethanol.
  Keep away from naked flames.

Kit component	Hazard contents	GHS symbol	Hazard phrases	Precaution phrases
Lysis buffer BLM	Guanidine hydrochloride	 Warning	H302/H315/ H319	P280/P305+P351+P338/P362/ P301+P312/P332+P313
Protease	Proteinase K, lyophilized	 Danger	H315/H319/ H334/ H335	P261/P305+P351+P338/ P342+P311
mag particle suspension BLM	Guanidine thiocyanate	 Danger	H314	P260/P303+P361+P353/ P305+P351+ P338/P310/P405
Wash buffer BLM 1	Guanidine thiocyanate	 Danger	H302+H312+ H332/H314/ H412	P260/P303+P361+P353/ P305+P351+P338/P310/ P405
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.

Do not use bleach for decontamination of liquid waste from extraction with mag maxi kit. Guandine thiocyanat (present in Lysis buffer BLM, mag particles and Wash buffer BLM 1) could form harmful compounds with bleach.

The waste which will be generated during extraction might still be infectious. Therefore it is recommended to handle it like infectious waste and dispose it with appropriate safety precautions.

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 for about 10 minutes.

Protease

Prepare the Protease by adding 56 mL of pure water to the vial of Protease. When not in use store the Protease at -20 °C. It is recommended to divide protease solution into suitable aliquots and store 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 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 **immediately**.

Number of samples	Vol. of Lysis buffer BLM	Vol. of Protease
1	220 µL	22 µL
20	4.4 mL	440 µL
72	15 mL	1.5 mL
96	21 mL	2.1 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

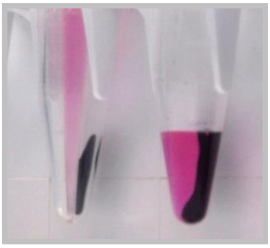
To prepare 100 mL of Wash buffer BLM 2 add 70 mL of acetone to 30 mL of Wash buffer BLM 2 concentrate. Mix well. Ensure the lid is closed tightly when the bottle is not in use to avoid evaporation. Alternatively you can use ethanol (96-100 %) instead of acetone for the preparation of Wash buffer BLM 2.

Manual protocol

1. Ensure blood samples **are well mixed prior** to starting the protocol. This is absolutely necessary to ensure effective re-suspension of DNA containing parts of the blood sample
2. Add 200 µL of **Lysis buffer BLM** and 20 µL of **Protease** to 200 µL of blood sample. Mix thoroughly, set pipette volume to 350 µL and pipette up and down 5 times
3. Incubate at 55 °C for 10 minutes then allow to cool to room temperature
4. Add 200 µL of **ethanol** to each sample
5. 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
6. Incubate for 2 minutes at room temperature to allow sufficient time for binding to occur. For agitating the sample during this time use a **shaker or vortex** periodically
7. Bring magnet into contact with the sample tubes. Wait for 1 minute at room temperature to allow the mag 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 720 µL of **Wash buffer BLM 1** and re-suspend the pellet. Mix thoroughly, set pipette volume to 650 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 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 720 µL of **Wash buffer BLM 2**
15. **Repeat steps 9 to 13** a second time with 720 µL of **Wash buffer BLM 2**
16. Dry the pellet at 55 °C for 10 minutes. Sample tubes must be **left open** to allow evaporation to occur
17. 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
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 mag 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 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.

Usage of the mag maxi kits on automated lab equipment

(liquid handling systems and/or magnetic particle manipulators)

The result of gDNA extraction strongly depends on the setup and technical features of the automated lab equipment in use.
In addition, the volumes given in the description of manual protocol have to be adapted to the technical specifications of the robot system in use and might be different (transport volume, air gap, excess volume).
Even in **case of this equipment is labelled as CE/IVD** device, the whole application (mag maxi kits and programmed method for DNA extraction) must be evaluated regarding the following parameters:

- Yield and quality of the DNA
- Cross-contamination of samples with reagents, other samples
- Transport of samples, sample management/tracking

LGC Genomics offers support in method validation/evaluation and how to adapt the protocol of this manual to automated systems (extraction@lgcgenomics.com).

Tips for automated protocol adaptation

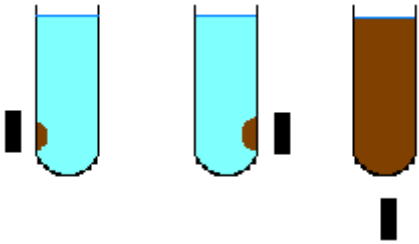
Follow the manual protocol as specified overleaf in respect to working steps, incubation times and volumes. Tips on automated mixing are given below:

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 BLM to **avoid frothing**
- Increase aspirate and dispense speeds when re-suspending pellets in wash buffers to ensure complete re-suspension.

Usage of magnetic particle manipulators (sep^{TM} boxes)

- sep boxes are computer driven magnetic particle collectors manufactured by LGC Genomics with active cooling and heating functionality
- **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.

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 (e.g. overnight)
	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 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
	Low protease activity	Make sure that protease solution is stored frozen (at -20 °C), protease tends to self digestion which deactivates the enzyme during longer storage at room temperature
	Heavily stained sample material	Check incubation time and temperature. 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}	Inefficient lysis	Check activity of protease
	Acetone carryover in eluate	Acetone has a maximum UV absorbance at 268 nm and a A_{260}/A_{280} ratio of 1.53. If this phenomenon occurs prolong the drying time to ensure all the acetone evaporates

www.lgcgroup.com/genomics
Email: genomics@lgcgroup.com

Ostendstr. 25 • TGS Haus 8 • 12459 Berlin • Germany
Tel: +49 (0)30 5304 2250 • Fax: +49 (0)30 5304 2201

Units 1 & 2 • Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ • UK
Tel: +44 (0)1992 470 757 • Fax: +44 (0)1438 900 670

100 Cummings Center • Suite 420H • Beverly • MA 01915 • USA
Tel: +1 (978) 232 9430 • Fax: +1 (978) 232 9435

43301/4.01-2014-05