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MagSep Blood gDNA Kit

Instructions for use

003 0451.902-02/012015

Table of contents

1 Operating instructions

1.1 Using this manual

- Before using this product for the first time, please read the entire manual.
- Read the software manual and the hardware manual of the epMotion.
- This manual is an intrinsic part of the product and should be kept in an accessible location.

1.2 Danger symbols and danger levels

The safety instructions of this operating manual indicate the following danger symbols and danger levels:

1.2.1 Danger symbols

 **General danger**	

1.2.2 Danger levels

DANGER	*Will* lead to severe injuries or death.
WARNING	*May* lead to severe injuries or death.
CAUTION	May lead to light to moderate injuries.
NOTICE	May lead to material damage.

1.3 Symbols used

Depiction	Meaning
1. 2.	Actions in the specified order
▸	Actions without a specified order
•	List
Text	Display text or software text
	Additional information

2 Product description

2.1 Main illustration

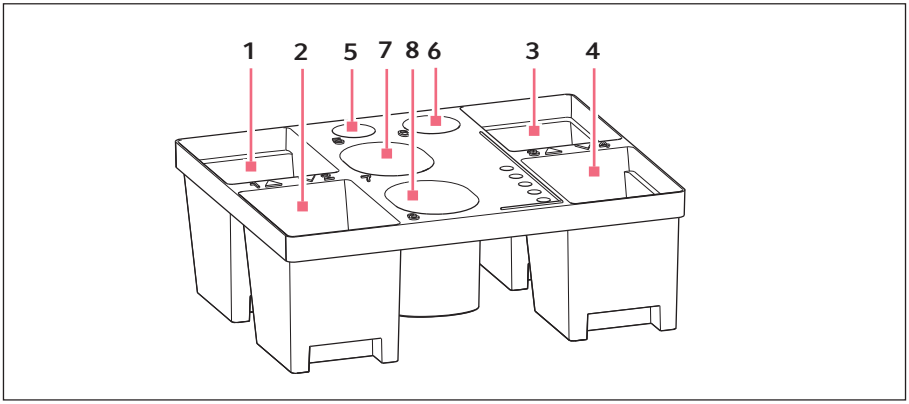


Fig. 2-1: MagSep Blood gDNA Kit

1 Blood BB (Binding Buffer)
2 Blood WB 1 (Wash Buffer)
3 Blood WB 2 (Wash Buffer)
4 Blood WB 3 (Wash Buffer)
5 Blood Beads
6 Blood ProK (Proteinase K)
7 Blood LB (Lysis Buffer)
8 Blood EB (Elution Buffer)

The numbering of the picture corresponds to the number of reagents in the MagSep Blood gDNA Kit.

2.2 Delivery package

Quantity	Description	Portion
4	Blood BB (Binding Buffer)	9 mL
4	Blood WB 1 (Wash Buffer)	23 mL
4	Blood WB 2 (Wash Buffer)	23 mL
4	Blood WB 3 (Wash Buffer)	23 mL
4	Blood Beads	0.8 mL
4	Blood ProK (Proteinase K)	30 mg
4	Blood LB (Lysis Buffer)	3 mL
4	Blood EB (Elution Buffer)	6 mL
4	Blood PB (Proteinase Buffer)	1.8 mL
4	Safe-Lock tubes 2.0 mL DNA LoBind, Pack of 50 tubes	
1	Manual for MagSep Blood gDNA Kit	

3 Safety

3.1 Intended use

MagSep Blood gDNA Kit components were developed, designed, distributed, and sold solely for research purposes. They are only suitable for in-vitro applications. No claim or representation is intended for its use for identifying any specific organism, or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

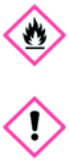
Rather, it is the responsibility of the user to verify the use of the MagSep Blood gDNA Kit for a specific application range, because the performance characteristic of this kit has not been verified for a specific organism.

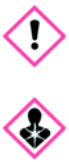
3.2 Warnings for intended use

DANGER! Danger due to reagents in the MagSep Blood gDNA Kit.
MagSep Kits contain hazardous contents.

- Wear your personal protective equipment.
- Follow the safety instructions in the Safety signs chapter.

3.3 Safety signs on the reagents

Component	Hazard content	GHS symbol			Hazard phrases	Precaution phrases	CAS
Blood LB (Lysis Buffer)	Guanidinium chloride 50% – 66%		GHS07	WARNING			50-01-1
Blood BB (Binding Buffer)	Ethanol 35% – 55% Sodium perchlorate 20% – 40%		GHS02 GHS07	WARNING			64-17-5 7601-89-0
Blood WB 1 (Wash Buffer)	Ethanol 20% – 35%		GHS02	WARNING			64-17-5
Blood WB 2 (Wash Buffer)	Ethanol 20% – 35%		GHS02	WARNING			64-17-5
Blood WB 3 (Wash Buffer)	Ethanol 55% – 75%		GHS02	DANGER			64-17-5

Component	Hazard content	GHS symbol			Hazard phrases	Precaution phrases	CAS
Blood ProK (Proteinase K)	Proteinase K		GHS07 GHS08	DANGER	H315 H317 H319 H334 H335	P261 P264 P271 P272 P280 P302+P352 P304+P340 P305+P351 +P338 P312 P333+P313 P337+P313 P342+P311 P363 P403+P233 P405	39450-01-6

3.3.1 Hazard phrases

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.

3.3.2 Precaution phrases

P261	Avoid breathing dust/fumes/gas/mist/vapors/spray.
P264	Wash thoroughly after handling.
P271	Use only outdoors or a well-ventilated area.
P272	Contaminated work clothing shoud not be allowed out of the workplace.
P280	Wear protective gloves/eye protection.
P302+352	IF ON SKIN: Wash with plenty of soap and water.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+ P338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing.
P312	Call a POISON CENTER of doctor/physician if you feel unwell.
P333+P313	If skin irritation or a rash occurs: Get medical advice/attention.
P337+P313	If eye irritation persists get medical advice/attention.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor/ physician.
P363	Wash contaminated clothing before reuse.
P403+P233	Store in a well ventilated place. Keep container tightly closed.
P405	Store locked up.

4 Operation

4.1 The basic principle

The MagSep Blood gDNA Kit purification procedure is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Whole blood is lysed with Blood LB (Lysis Buffer) and Blood ProK (Proteinase K). Magnetic beads are added after lysis incubation. Binding conditions, forcing DNA binding to the magnetic beads, are adjusted by adding Blood BB (Binding Buffer). After magnetic separation and removal of the supernatant, the paramagnetic beads are washed three times to remove contaminants and salt, followed by an ethanol evaporation step at high temperatures. Finally, highly purified DNA is eluted with low-salt Blood EB (Elution Buffer) and can be directly used for downstream applications.

The MagSep Blood gDNA Kit is optimized for use with the Eppendorf epMotion automated pipetting system.

4.1.1 Kit specifications

The MagSep Blood gDNA Kit is designed for rapid, automated, small-scale preparation of highly pure genomic DNA from whole blood using the 3D MagSep technology on the epMotion system. The obtained DNA is ready-to-use as a template for PCR, blotting, or any kind of enzymatic reaction. The actual processing time depends on the number of samples per batch.

Fresh blood, frozen blood or blood treated with EDTA, citrate or heparin, can be used. If leukocyte-rich materials such as buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS.

The procedure is optimized for a sample volume of 200 µL. Adjust smaller volumes to 200 µL with sterile PBS.

Blood Beads are highly reactive, superparamagnetic beads. The binding capacity is approximately 0.4 µg of gDNA per 1 µL of Blood Bead suspension, 1 µL of suspension contains 140 µg of beads.

4.2 Preparations

4.2.1 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulants (EDTA, citrate, or heparin) blood samples can be stored at +4 °C, or frozen.

Blood samples stored at +4 °C for up to 4 days will still allow for DNA isolation. However, DNA yield and quality will slowly decrease due to prolonged storage of blood samples under these conditions.

The highest DNA yields and quality are obtained from fresh blood.

4.2.2 Preparing the components of the MagSep Blood gDNA Kit

All buffers are delivered ready-to-use.

4.2.2.1 Handling of Blood Beads

A homogeneous distribution of the magnetic beads to each tube in the PrepRack is essential for high tube-to-tube consistency.

1. Before loading the tray onto the epMotion, make sure that the Blood Beads are completely resuspended.
2. Thoroughly shake the storage vial, or briefly place it on a vortexer.
3. To avoid an incorrect level measurement result from the optical sensor, make sure that there are no drops of Blood Beads in the cap or on the internal vial wall.
4. Place the vial correctly in the tray. Carefully push down the vial after reinserting it into the tray.

4.2.2.2 Preparing Blood ProK (Proteinase K) solution

We recommend preparing a fresh Proteinase K solution for each set of 24 reagents.

1. Add 1.5 mL of Blood PB (Proteinase Buffer) and mix gently to dissolve lyophilized Blood ProK (Proteinase K).
   This Proteinase K solution is stable at -20 °C for up to 6 months.
2. Before next use mix gently the Proteinase K solution.
3. Place Blood ProK (Proteinase K) in Position 6.

4.2.3 Starting process

Prerequisites

- epMotion is ready for operation.
- Make sure that all samples and all kit components are prepared as described.

1. Transfer 200 µL blood samples to 2 mL tubes provided with the kit, or use blood collection tubes.
2. Place the sample/elution tubes row-by-row in the racks, starting at position 1.
3. Vortex the Blood Beads.
4. Open the reagent bottles.
5. Empty the liquid waste.
6. Start the application Prep Assistant *MagSep Blood gDNA* at the EasyCon.
   The software assistant guides you through the set-up for automated nucleic acid purification.

4.3 Process

Procedure in the epMotion for

- Fresh or frozen blood treated with EDTA, citrate or heparin

Steps in procedure	Command	Description
Lyse sample	Reagent Transfer	Dispense 20 µL Blood ProK (Proteinase K)
	Thermomixer	Shake for 30 s, 1000 rpm.
	Reagent Transfer	Dispense 80 µL Blood LB (Lysis Buffer).
	Thermomixer	Shake for 10 min, 1200 rpm, 25 °C.
Bind NA to Blood Beads	Reagent Transfer	Dispense 300 µL Blood BB (Binding Buffer).
	Thermomixer	Shake for 30 s, 1200 rpm.
	Reagent Transfer	Dispense 25 µL Blood Beads.
	Thermomixer	Shake for 5 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.
1st wash	Reagent Transfer	Dispense 800 µL Blood WB 1 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.
2nd wash	Reagent Transfer	Dispense 800 µL Blood WB 2 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.
3rd wash	Reagent Transfer	Dispense 800 µL Blood WB 3 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.

Steps in procedure	Command	Description
Drying step	Thermomixer	Incubate for 7 min, 55 °C
	Thermomixer	Shake for 7 min, 1200 rpm, 55 °C
Elution	Reagent Transfer	Dispense 25 – 100 µL Blood EB (Elution Buffer).
	Thermomixer	Shake for 5 min, 1300 rpm, 55 °C.
	Separation	Separate for 2 min.
	Sample Transfer	Transfer eluted DNA.

4.4 Finishing

4.4.1 Completing the process

- Close the reagent bottles tightly after use. Alcohol may evaporate.

4.4.2 Elution procedures

Elution of purified genomic DNA can be carried out at a volume of 25 µL – 100 µL. For an optimal ratio of yield and concentration, perform the elution with a volume of 50 µL.

The eluate of heparin blood samples may contain PCR inhibitors. Frequently, heparin is not removed completely during the washing steps. To avoid problems in downstream PCR, dilute the eluate using an appropriate buffer (e.g., Blood EB).

For optimal performance of isolated DNA in subsequent downstream applications, we recommend storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

5 Troubleshooting

Problem	Cause	Solution
Salt ingredients of Blood LB (Lysis Buffer) or Blood BB (Binding Buffer) precipitate.	• Storage at low temperatures.	▶ Incubate the buffer at 40 °C and shake well.
Poor or no DNA yield.	• Wrong bottle arrangement in the tray.	▶ Check the position of all reagents in the tray.
	• Salt ingredients of Blood LB (Lysis Buffer) or Blood BB (Binding Buffer) precipitate.	▶ Incubate the buffer at 40 °C and shake well.
Low purity.	• Poor blood quality.	▶ Make sure that no blood clots are transferred to the well. ▶ Blood can be stored at 2 °C – 8 °C for up to 4 days. Freeze samples if stored for longer periods.
	• Salt ingredients of Blood LB (Lysis Buffer) or Blood BB (Binding Buffer) precipitate.	▶ Incubate the buffer at 40 °C and shake well.
Carry-over of beads	• High DNA concentration in the eluate.	▶ Increase the elution volume.
	• Not suitable or too much sample material.	▶ Decrease the used sample material.
Suboptimal performance of DNA in downstream applications.	• Wrong array in the tray.	▶ Check the position of all reagents in the tray.
	• Salt ingredients of Blood LB (Lysis Buffer) or Blood BB (Binding Buffer) precipitate.	▶ Incubate the buffer at 40 °C and shake well.
	• Ethanol evaporation from wash buffers.	▶ Close reagent bottles tightly after use.

Problem	Cause	Solution
Cross contamination.	• The *re-use tips* option is selected even though a SafeRack is not loaded.	▸ Be sure that a SafeRack is loaded. ▸ Be sure that the option *re-use tips* is not selected.
Faulty level detection.	• Vial is not correctly positioned.	▸ Make sure that the vial is placed on the bottom of the tray. Carefully press the vial down after reinserting it into the tray.
	• Bead solution on the internal vial wall.	▸ Make sure that there are no drops of bead solution in the cap or on the internal vial wall.

6 Transport, storage and disposal

6.1 Storage conditions

Reagent bottles must be stored tightly closed. Alcohol may evaporate.

Buffers and Beads of the MagSep Blood gDNA Kit	Stable at 18 °C – 25 °C for up to one year. Do not store below 18 °C, as salt ingredients may precipitate.
Blood ProK (Proteinase K) solution	Stable at -20 °C for up to 6 months.

7 Technical data

Technology	Magnetic bead technology
Sample material	<200 µL whole blood
Fragment size	300 bp – 30 kbp
Typical yield	2 µg – 8 µg
$A_{260/280}$	1.6 – 1.9
Elution volume	25 µL – 100 µL
Binding capacity	0.4 µg of gDNA per 1 µL of Blood Bead Suspension

8 Ordering information

8.1 Recommended pipette tips

epT.I.P.S. Motion SafeRack are intended for the reuse of tips within an epMotion application. They feature compartments which separates adjacent tips. The compartments prevent cross contamination of residual liquid in used tips. The use of epT.I.P.S. Motion SafeRacks is recommended when the *Re-use tips* option is selected in the software assistant.

Order no. (International)	Description
	epT.I.P.S. Motion Filter 50 µL 10 SafeRacks with 96 tips each
0030 014.618	PCR clean
	epT.I.P.S. Motion Filter 1 000 µL 10 SafeRacks with 96 tips each
0030 014.650	PCR clean

8.2 Alternative pipette tips

Order no. (International)	Description
	epT.I.P.S. Motion 50 µL 10 racks with 96 tips each
0030 014.405	Eppendorf Quality
0030 015.207	Sterile
	epT.I.P.S. Motion 1 000 µL 10 racks with 96 tips each
0030 014.480	Eppendorf Quality
0030 015.240	Sterile
	epT.I.P.S. Motion Filter 50 µL 10 racks with 96 tips each
0030 014.413	PCR clean
0030 015.215	PCR clean and Sterile
	epT.I.P.S. Motion Filter 1 000 µL 10 racks with 96 tips each
0030 014.499	PCR clean
0030 015.258	PCR clean and Sterile
	epT.I.P.S. Motion 50 µL 10 SafeRacks with 96 tips each
0030 014.600	Eppendorf Quality
	epT.I.P.S. Motion 1 000 µL 10 SafeRacks with 96 tips each
0030 014.642	Eppendorf Quality

8.3 MagSep Kits

Order no. (International)	Description
	MagSep Tissue gDNA Kit
0030 450.000	Reagent kit for DNA purification of 4 × 24 tissue and cell samples
	MagSep Blood gDNA Kit
0030 451.007	Reagent kit for DNA purification of 4 × 24 blood samples
	MagSep Viral DNA/RNA Kit
0030 452.003	Reagent kit for viral DNA/RNA purification of 4 × 24 cell free biological fluid samples.

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