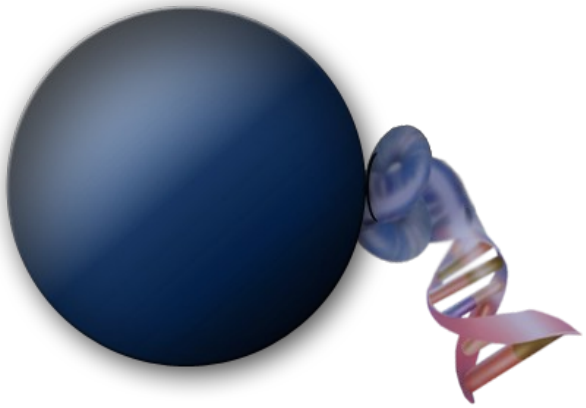
magtivio

MagSi-gDNA blood

Art.No.

MD60001 – MD61001 - MD62001



Product Manual

Version 1.0 | 07/08/2018

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1. General Information

1.1 Intended Use

This product is for Research Use Only (RUO). Not for drug, household or other uses.

The **MagSi-gDNA blood** kit is intended for manual and automated isolation of highly pure genomic DNA from whole blood. Processing time for the preparation of 96 samples is about 120 minutes. The kit requires no phenol/chloroform extraction or alcohol precipitation and eliminates the need for repeated centrifugation, vacuum filtration or column separation. It allows safe handling of potentially infectious samples, and is designed to avoid sample-to-sample cross-contaminations. The obtained DNA can be used directly for downstream applications such as PCR, blotting or any kind of enzymatic reaction.

The **MagSi-gDNA blood** kit is suitable for use with fresh or frozen blood treated with either EDTA or citrate. The procedure is optimized for 200 µL sample volumes. Reagent volumes can be scaled up or down to be used for different sample volumes.

The MagSi-DNA 3.0 COOH magnetic beads are optimized for use in isolating total DNA. The beads are easy to handle, have a high binding capacity and long suspension time enabling incubation without intensive mixing.

1.2 Kit specifications

The MagSi-gDNA blood kit provides reagents for extraction of 4–12 µg of total DNA from 200 µL whole blood samples with an A_{260}/A_{280} ratio of 1.7–2.0 and typical concentrations of 20–60 ng/µL. Depending on the elution volume used, concentrations of 10–160 ng/µL can be obtained.

The DNA obtained can be stored at 2-8°C. For long-term, storage at -20°C is recommended. To maintain the high-molecular weight nature of the isolated DNA, it is recommended to avoid multiple freeze-thaw cycles.

The kit can be processed completely at room temperature, but heating at 56°C during lysis and elution at 60-70°C may increase the yield up to 20%.

1.3 Basic principle

The protocol is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Whole blood is lysed under denaturing conditions by adding Lysis Buffer (LB) and Proteinase K. After lysis incubation, magnetic beads are added and binding conditions are adjusted by addition of Binding Buffer (BB) so that DNA binds to the magnetic beads. After magnetic separation and discard of the supernatant, the beads are washed three times to remove contaminants and salts. A drying step or the use of Wash Buffer III makes sure all traces of ethanol are removed. Finally, purified DNA is eluted with low-salt Elution Buffer (EB) and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	96 preps Art.No. MD60001	10 x 96 preps Art.No. MD61001
Lysis Buffer (LB)	20 mL	200 mL
Binding Buffer (BB)	50 mL	500 mL
Proteinase K	For 1 mL working solution	For 10 mL working solution
MagSi-DNA 3.0 COOH	2 mL	20 mL
Wash Buffer I (WB1)	2 x 80 mL	2 x 800 mL
Wash Buffer II (WB2)	80 mL	800 mL
Elution Buffer (EB)	20 ml	200 mL
Manual	1	1

For DNA extraction protocols without a drying step (Protocol 4.2), Wash Buffer III can be ordered separately (Art.No. MD70041). Please contact magtivio for further information.

2.2 Reagents, consumables and equipment to be supplied by the user

Reagents:

- ddH_2O (to reconstitute Proteinase K)

Consumables/equipment:

Protocol	Manual use	Automated use
Sample containers	1.5 or 2 mL microtubes	96 deep-well U-bottom microplate
Magnetic separation	MM-Separator M12 + 12, Art.No. MD90001	MM-Separator 96 DeepWell, Art.No. MDMG0013
Final container	1.5 or 2 mL microtubes	96-well U bottom microplate
Mixing	Vortexer	Thermoshaker
Heating	Water bath	

3. Kit usage

3.1 Storage Conditions

Kit components **Proteinase K** (lyophilized) and **MagSi-DNA 3.0 COOH** should be stored at 2-8°C. Store solutions of Proteinase K at -20°C. All other components of the MagSi-gDNA blood kit should be stored at room temperature (18-25°C). When stored under the conditions mentioned, the kit is stable for up to 1 year, but no longer than the expiry date on the label. Do not freeze!

3.2 Preparation of reagents

- All buffers are delivered ready-to-use.
- Reconstitute Proteinase K:
  - For **96 preps**, add 1 mL of **ddH_2O** to **Proteinase K**. Vortex for 5 seconds to dissolve. Store solutions of Proteinase K at -20°C.
  - For **10 x 96 preps**, add 10 mL of **ddH_2O** to **Proteinase K**. Vortex for 5 seconds to dissolve. Store solutions of Proteinase K at -20°C.
- Warm up the needed amount of Elution Buffer (EB) to 56°C (30-200 µL per sample).
- Buffers should be at room temperature.
- Blood samples should be thoroughly mixed.

3.3 Product use limitations

MagSi-gDNA blood is intended for research use only. Do not use them for other purposes than intended. The kit components can be used only once.

No guarantee is offered when taking different starting material. The kit is not validated for isolating DNA from for instance stool, tissue samples, bacteria, fungi or viruses, and is also not validated for the isolation of RNA.

The end-user has to validate the performance of the magtivio kits for any particular use, since the performance characteristics of the kits have not been validated for any specific application. magtivio kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

The product is intended for use by trained personnel. The DNA isolated can be used in most current genomic applications, such as: restriction digestion, cloning, Southern blotting, RFLP, PCR, HLA typing, sequencing.

Diagnostic results generated using the sample preparation procedure should only be interpreted with regard to other clinical or laboratory findings. Adequate controls should be used in each set of isolations, especially when used for diagnostic purposes.

3.4 Safety instructions

Take appropriate safety measures, such as wearing a suitable lab coat, disposable gloves, and protective goggles. Follow local legal requirements for working with biological materials.

More information is found in the safety data sheets (SDS), available at www.magtivio.com under each magtivio kit and kit component.

Infectious potential of liquid waste left over after using the MagSi-gDNA blood kit was not tested. Even though contamination of waste with residual infectious material is unlikely, it cannot be excluded completely. Therefore, liquid waste should be handled as being potentially infectious, and discarded according to local safety regulations.

3.5 Considerations

1. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.

2. Do not combine components of different kits unless the lot numbers are identical.

3. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.

4. Process only as many blood samples in parallel as the magnetic separator allows.

5. The elution can be done in smaller volumes of Elution Buffer (EB). Although this may result in higher DNA concentrations, overall yield may be lower. The yield may also be increased by prolonging the incubation time with pre-heated Elution Buffer.

6. The Elution Buffer (EB) does not contain EDTA (the end user may wish to use other elution buffers containing EDTA, or Tris and EDTA, though).

7. Blood samples may contain coagulates or precipitates. If these are visible, avoid aspirating them.

8. A complementary wash buffer for the MagSi-gDNA blood kit to replace the drying step can be ordered separately, Wash Buffer III (WB3), Art.No. MD70041. Wash Buffer III offers the advantages of obtaining increased purity, eliminates risks of residual alcohols and decreases the sample preparation time.

3.6 Magnetic Separation systems

The MagSi-gDNA blood kit has been designed for optimal use on the magtivio magnetic separator **MM-Separator M12 + 12** and **MM-Separator 96 DeepWell**.

The MM-Separator M12 + 12 (Art.No. MD90001) allows simultaneous processing of up to 12 samples in 1.5 mL or 2 mL microtubes. For automated protocols in 96 deep-well plates, use the MM-Separator 96 DeepWell (Art.No. MDMG0013).

For use with other magnetic separators, please contact the technical support at support@magtivio.com.

4. Protocols

4.1 Protocol for the isolation of DNA from whole blood

Before starting:

- *Check if Proteinase K was prepared according to section 3.2.*
- *Vortex magnetic beads thoroughly into a homogeneous suspension.*

1. Transfer 200 µL whole blood or up to 30 µL buffy coat into microtubes. If the volume is lower than 200 µL, bring the volume up to 200 µL with 1 x PBS buffer or distilled water.

2. Add 200 µL **Lysis Buffer (LB)** and 10 µL **Proteinase K**. Mix by pipetting up and down 5 times or by vortexing/shaking. Incubate the reaction tubes for 15 minutes at 56°C.

3. Add 500 µL of **Binding Buffer (BB)** and 20 µL of **MagSi-DNA 3.0 COOH**, mix the samples by pipetting up and down 5 times or by vortexing/shaking.

4. Incubate for 5 minutes at room temperature (RT). Place the tubes in a magnetic separator and wait for 2 minutes to collect the beads. Remove supernatants.

5. Add 800 µL **Wash Buffer I (WB1)** to the tubes. Mix by pipetting up and down 5 times or by vortexing/shaking. Place the tubes in a magnetic separator and wait for 1 minutes to collect the beads. Remove the supernatants.

6. Repeat step 5 one more time with 800 µL **Wash Buffer I (WB1)** and one time with 800 µL **Wash Buffer II (WB2)**.

7. Place the tubes for 5-10 minutes with opened lids to evaporate the ethanol completely.

8. Add 200 µL **Elution Buffer (EB)**, resuspend magnetic beads by pipetting up and down or by vortexing/shaking. Incubate at room temperature for 5-10 min.

9. Place the tubes in the MM-Separator M12 + 12 and incubate for 3 min. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates appear brownish, place the tubes on the MM-Separator M12 + 12 once again, incubate for 3 minutes and transfer the eluates to new tubes.*

    - *The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.*

    - *Heating of samples at 60-70°C during elution can increase DNA yields up to 20%. The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.*

4.2 Protocol for the isolation of DNA from whole blood using Wash Buffer III*

***Wash Buffer III (Art.No. MD70041) needs to be ordered separately.**

Before starting:

- *Check if Proteinase K was prepared according to section 3.2.*
- *Vortex magnetic beads thoroughly into a homogeneous suspension.*

1. Transfer 200 µL whole blood or up to 30 µL buffy coat into microtubes. If the volume is lower than 200 µL, bring the volume up to 200 µL with 1 x PBS buffer or distilled water.

2. Add 200 µL **Lysis Buffer (LB)** and 10 µL **Proteinase K**. Mix by pipetting up and down 5 times or by vortexing/shaking. Incubate the reaction tubes for 15 minutes at 56°C.

3. Add 500 µL of **Binding Buffer (BB)** and 20 µL of **MagSi-DNA 3.0 COOH**, mix the samples by pipetting up and down 5 times or by vortexing/shaking.

4. Incubate for 5 minutes at room temperature (RT). Place the tubes in a magnetic separator and wait for 2 minutes to collect the beads. Remove supernatants.

5. Add 800 µL **Wash Buffer I (WB1)** to the tubes. Mix by pipetting up and down 5 times or by vortexing/shaking. Place the tubes in a magnetic separator and wait for 1 minutes to collect the beads. Remove the supernatants.

6. Repeat step 5 one more time with 800 µL **Wash Buffer I (WB1)** and one time with 800 µL **Wash Buffer II (WB2)** .

7. With the tubes on the magnet, slowly add 800 µL **Wash Buffer III (WB3)*** to the tubes. Take care to disturb the pellet as little as possible! After 45 seconds, slowly remove the supernatants (carefully!). Do not exceed 90 seconds, this will decrease the yields significantly.

8. Add 200 µL **Elution Buffer (EB)**, resuspend beads by pipetting up and down or by vortexing/shaking. Incubate at room temperature for 5-10 min.

9. Place the tubes in the MM-Separator M12 + 12 and incubate for 3 min. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates appear brownish, place the tubes on the MM-Separator M12 + 12 once again, incubate for 3 minutes and transfer the eluates to new tubes.*

    - *The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.*

    - *Heating of samples at 60°C. during elution can increase DNA yields up to 20%. The DNA can be eluted with a lower volume of Elution Buffer (depending on the expected yield of genomic DNA). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of DNA is expected, the volume of Elution Buffer can be increased.*

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	Sample contains few or too many leukocytes	- Try using larger or smaller blood sample volumes
	Incomplete lysis	- Increase incubation time for lysis - Make sure lysis buffer does not contain precipitates - Blood sample may contain coagulates
	Inefficient binding to the magnetic particles	- Make sure lysis buffer and binding buffer do not contain precipitates - Use correct amount of all reagents - Increase mixing steps after adding Binding Buffer - Increase binding time - Mix sample during binding incubation
	Incomplete elution	- Temperature of lysis buffer may be incorrect - Drying of Wash Buffer II may have been incomplete - Try eluting twice with 100 µL Elution Buffer
	Incomplete collection of magnetic particles	- Prolong the time-to-magnet after binding step and washing steps
Degraded or sheared DNA	Incorrect storage of the sample material	- Sample should be harvested and stored properly - Avoid repeated thawing and freezing of blood sample
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Increase the evaporation time for Wash Buffer II
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that supernatants are completely removed. - Wash Buffers should be stored and used at RT - Repeat washing steps 2 and 3
	Magnetic beads remaining in the eluate	- Place the DNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

magtivio B.V.

Office:
Vlotstraat 2-4
6417 CB Heerlen (The Netherlands)
Tel: +31-(0)46-820 0206
Fax: +31-(0)46-410 6825
E-mail: info@magtivio.com

Lab & Production:
De Asselen Kuil 12a
6161 RD Geleen (The Netherlands)

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