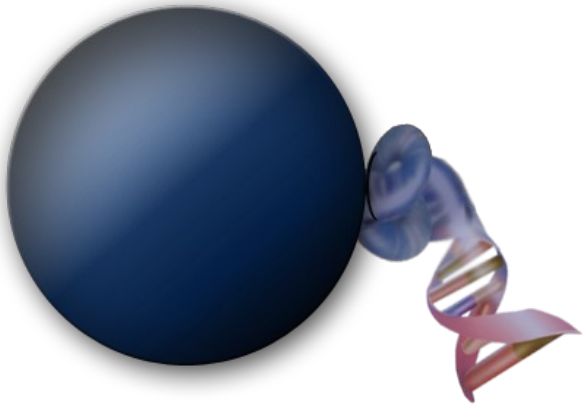
magtivio

MagSi-DNA Body Fluid

Art.No.

MDKT00140096

MDKT00140960



Product Manual

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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.

MagSi-DNA Body Fluid is intended for manual and automated isolation of genomic DNA from whole blood and saliva samples. Processing time for the preparation of 96 samples is about 40 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, or any kind of enzymatic reaction.

MagSi-DNA Body Fluid is suitable for use with fresh or frozen blood treated with either EDTA or citrate. It is possible to extract DNA from heparin treated whole blood, but this may result in inhibition of subsequent applications involving thermocycling.

The kit is also suitable for DNA extraction from fresh or preserved saliva.

MagSi-DNA BF beads are optimized for use in isolating total DNA. The beads are easy to handle and are supplied in a optimized storage buffer for increased suspension time.

The kit can be processed completely at room temperature.

1.2 Kit specifications

The kit provides reagents for extraction of 3–10 µg DNA from 200 µL whole blood samples with an A_{260}/A_{280} ratio of >1.7 and A_{260}/A_{230} ratio of >1.5, with 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 use, 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.

1.3 Basic principle

The protocol is based on reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. Cells are lysed under denaturing conditions by adding lysis buffer and Proteinase K. After incubation, magnetic beads are added and binding conditions are adjusted by addition of binding buffer 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 makes sure all traces of ethanol are removed. Finally, purified DNA is eluted with low-salt elution buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	96 preps MDKT00140096	10 x 96 preps MDKT00140960
Lysis Buffer U1	20 mL	200 mL
Binding Buffer U1	40 mL	400 mL
Proteinase K	For 1 mL working solution	For 10 mL working solution
MagSi-DNA BF	2 mL	20 mL
Wash Buffer I	2 x 80 mL	2 x 800 mL
Wash Buffer II	80 mL	800 mL
Elution Buffer	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:

- diH_2O (to reconstitute Proteinase K)

Consumables/equipment:

Protocol	Manual use	Automated use
Sample containers	1.5 or 2 mL microtubes	Recommended: Riplate®SW 96, PP, 2ml, (Ritter, 43001–0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0mL, (Thermo Scientific, Cat.No. 278752)
Magnetic separation	MM-Separator M12 + 12 P Art.No. MDMG0001	MM-Separator 96 DeepWell Art.No. MDMG0013
Final container	1.5 or 2 mL microtubes	96-well microplate
Mixing	Tube Vortexer	Microplate shaker (min. 1000 RPM)

3. Kit usage

3.1 Storage Conditions

Kit components **Proteinase K** (lyophilized) and **MagSi-DNA BF** should be stored at 2-8°C. Store ready solutions of Proteinase K at -20°C. All other components of the 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

- Reconstitute Proteinase K:
  - MDKT00140096 (96 preps), add **1 mL** of **diH_2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C
  - MDKT00140960 (10x96 preps), add **10 mL** of **diH_2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C
- Buffers should be at room temperature. Incubate Lysis Buffer U1, Binding Buffer U1 and Wash Buffer I at 37°C for 30 minutes if precipitates are visible. This can happen if storage temperatures are too low.
- Vortex magnetic beads thoroughly into a homogeneous suspension
- Samples should be thoroughly mixed before aliquotation

3.3 Product use limitations

MagSi-DNA Body Fluid is intended for Research Use Only. Do not use for other purposes than intended. The kit components can be used only once.

No guarantee is offered when using sample material other than whole blood or saliva. 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 kit 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 isolated DNA can be used in most current genomic applications, such as: restriction digestion, PCR, 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-DNA Body Fluid 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. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.

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

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

5. The elution can be done in smaller volumes of Elution Buffer. Although this may result in higher DNA concentrations, overall yield may be lower. The yield may also be increased by prolonging the incubation time, and with pre-heated Elution Buffer (56°C).

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

7. Avoid using blood samples containing coagulates or precipitates, as this may result in poor quality DNA.

8. The kit is compatible with whole blood treated with EDTA and citrate. Heparin is co-isolated and may interfere with subsequent DNA analyses.

9. When extracting DNA from saliva, eluates may appear turbid due to fine particulates from saliva. Although these particulates typically do not cause inhibition in subsequent PCR analyses, the particulates can be easily removed by a brief centrifugation step.

10. A complementary wash buffer for MagSi-DNA Body Fluid to replace the drying step can be ordered separately, Wash Buffer III (WB3), Art.No. MD70041. Wash Buffer III eliminates risks of inhibition by residual alcohols, and may increase DNA purity.

11. It may occur that a small amount of beads is accidently transfered with the final DNA sample, but most likely this will not inhibit subsequent applications. However, if desired another separation step can be performed to remove the beads.

3.6 Magnetic Separation systems

MagSi-DNA Body Fluid has been designed for optimal use on the magtivio magnetic separators **MM-Separator M12 + 12 P** and **MM-Separator 96 DeepWell**. MM-Separator M12 + 12 P 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.

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

MagSi-DNA Body Fluid is compatible with KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Protocols and consumables are available on request.

4. Protocols

4.1 DNA extraction from 200 µL whole blood or saliva

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 sample 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 U1** and 10 µL **Proteinase K**. Mix by pipetting up and down 5 times or by vortexing. Incubate samples for 10 minutes under shaking.
3. Add 400 µL **Binding Buffer U1** and 20 µL **MagSi-DNA BF**. Vortex the samples and incubate for 5 min under shaking.
4. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
5. Add 800 µL **Wash Buffer I** to the tubes. Mix by vortexing shortly and wait for 1 min. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
6. Repeat step 5 one more time with 800 µL **Wash Buffer I** and one time with 800 µL **Wash Buffer II**.
7. Place the tubes for 10 min with opened lids to evaporate the ethanol completely.
8. Add 200 µL **Elution Buffer**, resuspend magnetic beads by pipetting up and down or by vortexing. Incubate the reaction tubes for 5 minutes under shaking.
9. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates appear turbid, briefly centrifuge the samples and carfefully 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.*

4.2 DNA extraction from 200 µL whole blood or saliva 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 sample 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 U1** and 10 µL **Proteinase K**. Mix by pipetting up and down 5 times or by vortexing. Incubate samples for 10 minutes under shaking.
3. Add 400 µL **Binding Buffer U1** and 20 µL **MagSi-DNA BF**. Vortex the samples and incubate for 5 min under shaking.
4. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
5. Add 800 µL **Wash Buffer I** to the tubes. Mix by vortexing shortly and wait for 1 min. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
6. Repeat step 5 one more time with 800 µL **Wash Buffer I** and one time with 800 µL **Wash Buffer II**.
7. With the tubes on the magnet, add 800 µL **Wash Buffer III**. Wait for 30 seconds and remove the supernatant again. Do not resuspend beads and do not exceed 60 seconds as this may cause early DNA elution. When using the kit manually, it is recommended to not treat samples with Wash Buffer III simultaneously.
8. Add 200 µL **Elution Buffer**, resuspend magnetic beads by pipetting up and down or by vortexing. Incubate the reaction tubes for 5 minutes under shaking.
9. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates appear turbid, briefly centrifuge the samples and carfefully 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.*

4.3 DNA extraction from 500 µL Oragene saliva sample

Before starting:

- *Incubate the samples at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours to ensure that DNA is adequately released and nucleases are permanently inactivated. This incubation step may be performed at any time after the sample is collected and before it is purified. The samples can also be incubated overnight for convenience.*
- *Mix the sample in the Oragene kit by inversion and gentle shaking for a few seconds to ensure that viscous samples are properly mixed.*
- *Vortex magnetic beads thoroughly into a homogeneous suspension.*

1. Transfer 500 µL Oragene sample into microtubes.
2. Add 400 µL **Binding Buffer U1** and 20 µL **MagSi-DNA BF**. Vortex the samples and incubate for 5 min under shaking.
3. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
4. Add 800 µL **Wash Buffer I** to the tubes. Mix by vortexing shortly and wait for 1 min. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
5. Repeat step 5 one more time with 800 µL **Wash Buffer I** and one time with 800 µL **Wash Buffer II**.
6. Place the tubes for 10 min with opened lids to evaporate the ethanol completely.
7. Add 200 µL **Elution Buffer**, resuspend magnetic beads by pipetting up and down or by vortexing. Incubate the reaction tubes for 5 minutes under shaking.
8. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

- *If the transferred eluates appear turbid, briefly centrifuge the samples and carfefully 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.*

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	Sample contains few leukocytes	- Try using larger or smaller blood sample volumes
	Incomplete lysis	- Increase incubation time for lysis - Make sure Lysis Buffer U1 does not contain precipitates - Blood sample may contain coagulates
	Inefficient binding to the magnetic particles	- Make sure Lysis Buffer U1 and Binding Buffer U1 do not contain precipitates - Use correct amount of all reagents - Increase mixing steps after adding Binding Buffer U1 - Mix sample during binding incubation
	Incomplete elution	- Drying of Wash Buffer II may have been incomplete - Try eluting twice with 100 µL Elution Buffer
Degraded or sheared DNA	Incorrect storage of the sample material	- Sample should be collected and stored properly - Avoid repeated freezing and thawing of blood sample
Purified DNA samples are turbid	Saliva sample contains solid particulates	- Centrifuge briefly and transfer samples to a new container
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Increase the drying time to 15 minutes - Use protocol 4.2 with Wash Buffer III
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that supernatants are properly removed. - Wash Buffers should be stored and used at RT
	Magnetic beads remaining in the eluate	- Place the DNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

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