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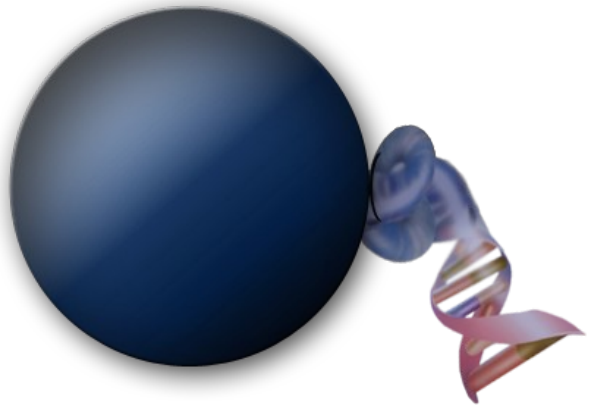
MagSi-DNA Body Fluid

Art.No.

MDKT00140096

MDKT00140960



Product Manual

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Revision history		
Revision	Release date	Remarks
1.0	06/02/2019	Initial release
9.0	08/03/2022	Updated sections 2.1, 3.1, 3.2 and 4.3, layout changes

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1. General Information

1.1 Intended Use

MagSi-DNA Body Fluid is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

The kit 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-BF9 beads are optimized for use in isolating total DNA. The beads are easy to handle and are supplied in an optimized storage buffer for increased suspension time.

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 obtained DNA should be used immediately after extraction. Storage at <-20°C is recommended for later analysis. To maintain the high-molecular weight nature of the isolated DNA, it is recommended to avoid multiple freeze-thaw cycles.

1.3 Basic principle

Samples are lysed under denaturing conditions by adding Lysis Buffer U1 and Proteinase K. After incubation, MagSi-BF9 beads are added and binding conditions are adjusted by addition of Binding Buffer U1 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	20 mg (for 1.1 mL working solution)	200 mg (for 11 mL working solution)
MagSi-BF9	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
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2.2 Reagents, consumables and equipment to be supplied by the user

Reagents:

- molecular biology grade (nuclease free) water to reconstitute Proteinase K

Consumables and equipment for manual use or automated processing on liquid handling robots

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)

Consumables for processing on the PurePrep 96 System or KingFisher™ Flex instrument

Product	Art. No.	Contents
2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	MDPL00200060	60 pieces
200 µL square-well Elution Plate for KingFisher™/PurePrep 96	MDPL00190060	60 pieces
96 well Tip-Comb for KingFisher™/PurePrep 96	MDPL00210060	60 pieces

3. Kit usage

3.1 Storage Conditions

All components of the kit should be stored at room temperature (18-25°C). Store working solutions of reconstituted Proteinase K at -20°C. When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Preparation of reagents

- Reconstitute Proteinase K:

  MDKT00140096 (96 preps), add **1.1 mL** of **diH_2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C

  MDKT00140960 (10x96 preps), add **11 mL** of **diH_2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C

- If there is any precipitate present in the buffers, warm the buffer to 25-37°C to dissolve the precipitate before use.

- Immediately before use, resuspend MagSi-BF9 beads by vortexing for 20 seconds. If preferred, MagSi-BF9 beads can be premixed with Binding Buffer U1 for simultaneous addition to samples. The mixture must used on the day of preparation, and mixed well by vortexing before transfer to samples. For each sample, prepare Binding Buffer / Beads premix:

Binding Buffer U1	400 µL
MagSi-BF9	20 µL
Total	420 µL

- Samples should be thoroughly mixed before use.

3.3 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 info@amsbio.com

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.

3.4 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.

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, some 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. 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.

3.5 Magnetic Separation systems

MagSi-DNA Body Fluid has been designed for use on the MM-Separator 96 DeepWell and MM-Separator M12 + 12 P. The MM-Separator M12 + 12 P (Art.No. MDMG0001) allows simultaneous processing of up to 12 samples in 2 mL microcentrifuge tubes. For processing in 96 deepwell plates, use the MM-Separator 96 DeepWell (Art.No. MDMG0013).

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

MagSi-NA Pathogens is compatible with the PurePrep 96 System and the KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Information of use on these instruments is described in sections 4.3 and 4.4. Software protocol files are available on request.

3.6 Shaker settings

The speed settings for the microplate shaker described in the protocols that follow were defined with a specific instrument and microplate. When first using a plate shaker for incubation steps, the speed settings have to be set carefully for each specific plate to prevent cross contamination and spillage. Setting the speed of the shaker can be done by loading a microplate with a volume of dyed water equal to the working volume during each step, and step-wise increasing the shaker speed until droplets are observed on the surface of the plate. Set the shaker speed lower again.

3.7 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. These 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 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.

4. Protocols

4.1 Manual 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 a deepwell microplate or 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**. Incubate on a shaker for **10 min at 1000 RPM**. Spin down briefly to collect any sample from the microtube lid.

3. Add **20 µL MagSi-BF9** beads and **400 µL Binding Buffer U1** 🟢. Mix and incubate on a shaker for **5 min at 1000 RPM**. Spin down briefly to collect any sample from the microtube lid.

4. Place the samples on the magnetic separator and wait at least 1 minute to collect the beads. Remove supernatants without disturbing the attracted magnetic bead pellet.

5. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** 🔵 to the tubes. Resuspend the beads by incubation of the samples on a shaker for **1 min at 1000 RPM** (alternatively pipette the wash buffer up and down until the beads are resuspended completely). Place the samples in a magnetic separator and wait at least 1 minute to collect the beads. Remove the supernatants without disturbing the attracted bead pellet.

6. Repeat step 5 one more time with **800 µL Wash Buffer I** 🔵 and one more time with **800 µL Wash Buffer II** 🟡**.**

7. Dry the beads on air for **10 min** to evaporate the ethanol completely. If necessary, briefly spin down and remove any buffer residues before drying the attracted magnetic beads.

8. Remove the samples from the magnetic separator and add **50-200 µL Elution Buffer** 🟠**.** Resuspend the attracted magnetic beads by repeated pipetting up and down. **Incubate** on a shaker for **10 min at 1000 RPM.**

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 carefully transfer the eluates.*

    - *If the transferred eluates contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates.*

    - *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 Manual 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-BF9**. Incubate on a shaker for **5 min at 1000 RPM**.
3. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
4. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** 🔵 to the tubes. Incubate on a shaker for 1 min at 1000 RPM. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
5. Repeat step 4 one more time with **800 µL Wash Buffer I** 🔵 and one time with **800 µL Wash Buffer II** 🟡.
6. Dry the beads on air for **10 min** to evaporate the ethanol completely.
7. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer** 🟠. Incubate on a shaker for 5 min at 1000 RPM.
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 carefully 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 Protocol for the PurePrep 96 System

4.3.1 PurePrep 96 software protocol file

Please contact info@amsbio.com for the most recent software method files. We provide the corresponding files for directupload on the PurePrep 96 System. Refer to the PurePrep 96 user manual regarding the upload procedure of the
supplied software files to the instrument.

4.3.2 Sample lysis (offline)

1. Add **10 µL Proteinase K** to each well of the **Sample Plate**. Dispense the Proteinase K with contact to the well bottom. Check optically if the Proteinase K is dispensed into each well.

2. Add 200 µL of blood or saliva sample to each well of the **Sample Plate**. In case of using less than 200 µL sample fill-up the samples with 1 x PBS buffer or water.

3. Add **200 µL Lysis Buffer U1** . Mix immediately and incubate on a shaker for **10 min with shaking at 1000 RPM**.

4.3.3 Preparation of processing plates

Initial Plate filling instructions:

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Empty, for loading Tip-Comb only	N/A	1
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Proteinase K Blood or saliva sample Lysis Buffer U1	10 µL 200 µL 200 µL	2
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	3
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	4
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL	5
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	100 µL	8

Suitable plates can be purchased at AMSBIO (see section 2.2). We strongly recommend using only the plates which are intended to use on the PurePrep 96 System. Using unsuitable plates may result in extraction failure or instrument damage.

To be added after the initial lysis step:

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	MagSi-BF9 beads Binding Buffer U1	20 µL 400 µL	2

4.3.4 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Prepare "Wash Plate 1" and "Wash Plate 2" with **Wash Buffer I.** Add **800 µL Wash Buffer I** to each well of the corresponding deep-well plates.
2. Prepare "Wash Plate 3" with **Wash Buffer II**. Add **800 µL Wash Buffer II** to each well of the corresponding deep-well plate.
3. Prepare "Elution Plate" with **Elution Buffer**. Add **150 µL Elution Buffer** to each well of the corresponding square-well elution plate.
4. Equilibrate samples to room temperature. Mix well using a suitable tube roller mixer or similar device.
5. Add **10 µL Proteinase K** to each well of the "Sample Plate". Dispense the Proteinase K with contact to the well bottom. Check optically if the Proteinase K is dispensed into each well.
6. Add **200 µL of blood or saliva sample** to each well of the "Sample Plate". In case of using less than 200 µL sample fill-up the samples with 1 x PBS buffer or water.
7. Add **200 µL Lysis Buffer U1** to each well of the "Sample Plate". Mix by pipetting up and down until a homogeneous mixture is visible (typically 3 – 4 times).
8. Mix immediately and incubate on a shaker for **10 min with shaking at 1000 RPM**.
9. Add **20 µL of MagSi-BF9 beads** to each well of the "Sample Plate".
10. Add **400 µL of Binding Buffer U1** to each well of the "Sample Plate".
11. Switch on the PurePrep 96 System and select the protocol from the user defined protocols
12. Load all plates to the PurePrep 96 instrument on indicated positions, see section 4.3.3 (right-most column). Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions.

    Make sure that the plates are loaded in the correct orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable. Make sure that the plates are fixed to the positions by the clamps.
13. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
14. At the end of the run remove all plates from the instrument

4.4 Protocol for the KingFisher Flex™ Magnetic Particle Processor

4.4.1 KingFisher BindIt software protocol

Please contact info@amsbio.com for the most recent BindIt software method files. We provide the corresponding files fordirect upload on the KingFisher™ magnetic particle processors. A PDF description of the method file is included.Refer to the BindIt software manual regarding the upload procedure of the supplied software files to the
instrument.

4.4.2 Preparation of processing plates

Initial plate filling for instrument set-up:

Plate	Type*)	Reagent	Volume
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Proteinase K Blood or saliva sample Lysis Buffer U1	10 µL 200 µL 200 µL
Wash Plate 1	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 2	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 3	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	150 µL
Tip plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Empty, for loading Tip-Comb only	N/A

To be added after the lysis step on the KingFisher Flex magnetic particle processor

Plate	Type*)	Reagent	Volume
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	MagSi-BF9 beads Binding Buffer U1	20 µL 400 µL

Suitable plates can be purchased at AMSBIO (see section 2.2). We strongly recommend using only the plates which are intended to use on the KingFisher magnetic particle processor. Using unsuitable plates may result in extraction failure or instrument damage.

4.4.3 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Equilibrate samples to room temperature. Mix well using a suitable tube roller mixer or similar device.

2. Add **10 µL Proteinase K** to each well of the "Sample Plate". Dispense the Proteinase K with contact to the well bottom. Check optically if the Proteinase K is dispensed into each well.

3. Add **200 µL blood or saliva sample** to each well of the "Sample Plate". In case of using less than 200 µL sample fill-up the samples with 1 x PBS buffer or water.

4. Add **200 µL Lysis Buffer U1** 🔴 to each well of the "Sample Plate". Mix by pipetting up and down until a homogeneous mixture is visible (typically 3 – 4 times).
   Optionally: place the plate on a plate shaker and shake while preparing the remaining plates.

5. Prepare "Wash Plate 1" and "Wash Plate 2" with **Wash Buffer I.** Add **800 µL Wash Buffer I** 🔵 to each well of the corresponding deep-well plates.

6. Prepare "Wash Plate 3" with **Wash Buffer II**. Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.

7. Prepare "Elution Plate" with **Elution Buffer**. Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.

8. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols.

9. Start the protocol.

10. Load the plates to the instrument, following the instructions on the instrument display. Order of plates start with the tip plate and ends with the sample plate. Sample lysis starts immediately after loading the sample plate to the instrument.

    Make sure that all plates are inserted in the same orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable.

11. After approx. 12 min the instrument stops and moves the Sample Plate to the plate loading position. Remove the plate and add **20 µL MagSi-BF9** and **400 µL Binding Buffer U1** 🟢

12. Reload the plate to the instrument and continue the purification process.

13. At the end of the method remove all plates from the instrument. Follow the information on the instrument display.

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
	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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