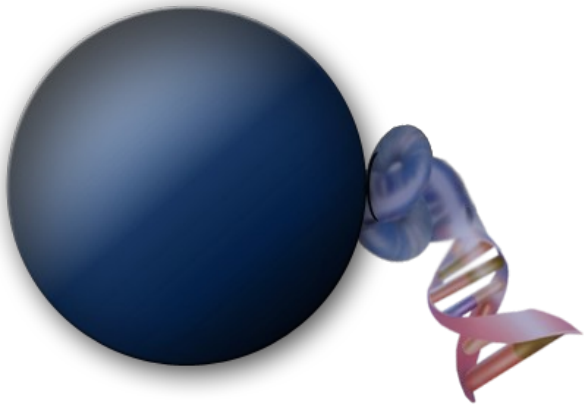
magtivio

MagSi-DNA Animal

Art.No.
MDKT00150096
MDKT00150960



Product Manual

Version 4.0 | 25/02/2020

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1. General Information

1.1 Intended Use

MagSi-DNA Animal 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 veterinary samples such as blood, semen, hair, ear punches or swabs. Processing time for DNA extraction from 96 samples is about 30 minutes. The kit requires no phenol/chloroform extraction or ethanol precipitation and eliminates the need for repeated centrifugation, vacuum filtration or column separation. It allows safe handling of samples, and is designed to avoid sample-to-sample cross-contaminations.

MagSi-DNA Animal is suitable for automation on most liquid handling robots. The total processing time depends on the throughput and configuration of the instrument. The beads are easy to handle, have a high binding capacity and enable incubation without intensive mixing.

1.2 Kit specifications

The kit provides reagents for extraction of DNA from 96 or 10x96 samples. Purified DNA samples 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 freeze-thaw cycles.

1.3 Principle of operation

Cell contents are released with Lysis Buffer U1 containing chaotropic salts and detergents. Lysed samples should be cleared by centrifugation in order to remove cellular debris. By adding MagSi-AG IV magnetic beads and adjusting binding conditions by addition of Binding Buffer U1, DNA binds to the magnetic beads while leaving impurities in solution. After magnetic separation and removal of the supernatant, the beads are washed three times to remove any residual contaminants and potential PCR inhibitors. A drying step makes sure all traces of ethanol are removed. Finally, purified DNA is eluted off the beads with 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	40 mL	400 mL
Lysis Buffer TS	40 mL	400 mL
Lysis Buffer VT	40 mL	400 mL
Binding Buffer U1	50 mL	500 mL
MagSi-AG IV	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:

- 80% ethanol
- Proteinase K (10 mg/mL)
- Dithiothreitol (DTT) (1 M) (optional)
- RNase (10 mg/mL) (optional)

Consumables and equipment for manual or automated processing

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 KingFisher Flex instrument

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

3. Kit usage

3.1 Storage Conditions

All components of the MagSi-DNA Animal kit can be stored at room temperature (18-25°C). The kit is stable for up to 1 year, but no longer than the expiry date on the label.

3.2 Lysis Buffers

MagSi-DNA Animal includes 3 lysis buffers, which can be used as following:

Product	Samples types
Lysis Buffer U1 🔴	blood, saliva, swabs, semen, tissue preservation buffer (Allflex)
Lysis Buffer TS 🔵	Tissue, hair roots
Lysis Buffer VT 🟤	Blood cards

3.3 Preparation of reagents

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

Small scale protocol

- Prepare a lysis Working Solution by adding Proteinase K and DTT to Lysis Buffer U1 as following:
  - Per 200 µL Lysis Buffer U1 🔴, Lysis Buffer TS 🔵 or Lysis Buffer VT 🟤, add 10 µL Proteinase K (10 mg/mL), 10 µL DTT (1 M) (optional), and 5 µL RNase A (10 mg/mL) (optional)
  - Prepare a little more Lysis Working Solution than needed due to loss during pipetting (e.g. for 96 extraction prepare solution for 100 extractions.

Full scale protocol

- Prepare a lysis Working Solution by adding Proteinase K and DTT to Lysis Buffer U1 as following:
  - Per 400 µL Lysis Buffer U1 🔴, Lysis Buffer TS 🔵 or Lysis Buffer VT 🟤, add 20 µL Proteinase K (10 mg/mL), 20 µL DTT (1 M) (optional), and 10 µL RNase A (10 mg/mL) (optional)
  - Prepare a little more Lysis Working Solution than needed due to loss during pipetting (e.g. for 96 extraction prepare solution for 100 extractions.

Magnetic bead suspension

- Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds.

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 Animal 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. Depending on animal species and sample type, the volume of the Lysis Buffer U1 can be optimized.

2. Most animal samples require the use of Proteinase K to digest proteins.

3. Some samples, such as semen, require DTT to disrupt disulfide bonds of proteins.

4. If samples contain large amounts of RNA, it is recommend to add RNase A (10 mg/mL) to the lysis mixture before incubation.

5. Elution can be performed at room temperature. Yields may be increased if elution is performed at 60°C. In most consumables elution can be carried out in ≥50 µL. Do not use less than the minimum working volume of the container used as it is essential to completely submerge the beads in elution buffer during the elution step and in order to allow magnetic separation. For some separators and sample containers, higher or lower elution volumes may be needed to contact the whole magnetic bead pellet. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.

6. Lysis efficiency and DNA yield are highly dependent on the sample type. Different volumes of Lysis Buffer U1 can be used to increase DNA yields. Conditions for binding have to be adjusted by taking a volume of Binding Buffer U1 that is at least 1.6 times the volume of lysate transferred after centrifugation.

3.6 Magnetic Separation systems

MagSi-DNA Animal 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 support@magtivio.com.

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

3.7 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.

3.8 Product use limitations

The MagSi-DNA Animal Kit is intended for research use only. Do not use for other purposes than intended. The kit components can be used only once. Do not combine components of different kits unless the lot numbers are identical. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents. Process only as many samples in parallel as the magnetic separator allows.

4. Protocols

4.1 Suggested sample lysis recommendations

The specified volumes can be considered as a starting point for DNA extraction. Depending on the individual sample materials and sample conditions the sample input volume/weight may need to be optimized.

Blood samples: 20 µL blood

Semen samples: 30 µL semen. Including DTT in the lysis mixture is mandatory. Incubate at least 3 h or overnight

Tissue sample: small piece of tissue / biopsy / ear punch, approx. 4 mm^3 use less than 10 mg. Incubate at least 3 h or overnight.

Saliva samples / swab samples (e.g. DNA Genotek´s PERFORMAgene PG-100 swab kit): 100 µL

Hair roots: 8 hairs. Including DTT in the lysis mixture is mandatory. Incubate at least 3 h or overnight.

For use of the full scale procedure use the doubled volumes for sample and reagents.

4.2 Manual DNA extraction from animal samples (small scale protocol)

Before starting:

- *Prepare Lysis Working Solution according to section 3.2*
- *Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds*

1. Transfer the sample to a deepwell microplate or microtube. See section 4.1 for suggested sample volumes. To each sample, add **200 µL Lysis Working Solution** and incubate the samples at **56°C** for **3 hours**.
2. Centrifuge for **15 min (>6.000 x g)** to pellet contaminants and cell debris. Transfer **150 µL cleared lysate** to a deepwell microplate or microtube.
3. Add **250 µL Binding Buffer U1** and **10 µL MagSi-AG IV**. Incubate on a shaker for 5 min at 1000 RPM.
4. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
5. Remove the sample plate from the magnetic separator and add **400 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.
6. Repeat step 6 one more time with **400 µL Wash Buffer I** and one time with **400 µL Wash Buffer II**
7. Dry the beads on air for **10 min** to evaporate the ethanol completely.
8. 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.

9. Place the samples on the 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 contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.*

    - *The DNA can be eluted with different volumes of Elution Buffer (depending on the required volume for subsequent analysis).*

4.3 Manual DNA extraction from animal samples (full scale protocol)

Before starting:

- *Prepare Lysis Working Solution according to section 3.2*
- *Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds*

1. Transfer the sample to a deepwell microplate or microtube. When using liquid sample, use up to 200 µL. To each sample, add **400 µL Lysis Working Solution** and incubate the samples at **56°C** for **3 hours**.
2. Centrifuge for **15 min (>6.000 x g)** to pellet contaminants and cell debris. Transfer **300 µL cleared lysate** to a new deepwell microplate or microtube.
3. Add **500 µL Binding Buffer U1** and **20 µL MagSi-AG IV**. Incubate on a shaker for 5 min at 1000 RPM.
4. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
5. 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.
6. Repeat step 6 one more time with **800 µL Wash Buffer I** and one time with **800 µL Wash Buffer II**
7. Dry the beads on air for **10 min** to evaporate the ethanol completely.
8. 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.
9. Place the samples on the 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 contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.*
    - *The DNA can be eluted with different volumes of Elution Buffer (depending on the required volume for subsequent analysis).*

4.4 DNA extraction from animal samples on the KingFisher Flex™

4.4.1 KingFisher BindIt software protocol (small scale protocol)

Please contact magtivio for the most recent BindIt software method files. We provide the corresponding files for direct 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 Sample lysis

1. Transfer the sample to a deepwell microplate or microtube. To each sample, add **200 µL Lysis Working Solution** and incubate the samples at **56°C** for **3 hours**.
2. Centrifuge for **15 min (>6.000 x g)** to pellet contaminants and cell debris. Transfer 150 µL cleared lysate to a 2 ml Deepwell Plate with square wells for KingFisher™.

4.4.3 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™	Lysate Binding Buffer U1 MagSi-AG IV	150 µL 250 µL 10 µL
Wash Buffer I - 1	2 ml Deepwell Plate with square wells for KingFisher™	Wash Buffer I	400 µL
Wash Buffer I - 2	2 ml Deepwell Plate with square wells for KingFisher™	Wash Buffer I	400 µL
Wash Buffer II	2 ml Deepwell Plate with square wells for KingFisher™	Wash Buffer II	400 µL
Elution Buffer	200 µL square-well Elution Plate for KingFisher™	Elution Buffer	150 µL
Tip plate	2 ml Deepwell Plate with square wells for KingFisher™	Empty, for loading Tip-Comb only	N/A

*) We strongly recommend to use only the plates which are intended to use on the KingFisher magnetic particle processor. Using insuitable plates may result in extraction failure or instrument damage.

4.4.4 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 one plate for the binding step with MagSi-AG IV and Binding Buffer VG. To each well of the Sample Plate already containing 150 µL lysate, dispense 10 µL **MagSi-AG IV** magnetic beads and 250 µL **Binding Buffer U1** 🟢.

2. Prepare one plate for the 1st wash step with **Wash Buffer I.** Add 400 µL **Wash Buffer I** 🔵 to each well of the corresponding deep-well plate.

3. Prepare one plate for the 2nd wash step with **Wash Buffer I**. Add 400 µL **Wash Buffer I** 🔵 to each well of the corresponding deep-well plate.

4. Prepare one plate for the 3rd wash step with **Wash Buffer II**. Add 400 µL **Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.

5. Prepare one plate for **Elution Buffer**. Add 150 µL **Elution Buffer** 🟠 to each well of the corresponding Elution plate.

6. Switch on the KingFisher Flex magnetic particle processor and select the **"MagSi-DNA-Animal-150"** protocol from the user defined protocols

7. Start the protocol.

8. Load the plates to the instrument, following the instructions on the instrument display. The order of plates starts with the tip plate and ends with the sample plate.

   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.

9. At the end of the method remove all plates from the instrument. Follow the instructions on the instrument display.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	Sample contains too low or too high amounts of animal material	- Try using larger or smaller amounts of animal material
	Incomplete lysis	- Increase incubation time for lysis - Make sure Lysis Buffers do not contain precipitates
	Inefficient binding to the magnetic particles	- Use correct amounts of all reagents - Make sure the shaker speed is set correctly - Increase binding time
	Incomplete elution	- Increase drying time for evaporation of ethanol - Increase elution time from 5 to 10 minutes - Preheat Elution Buffer to 60°C before use - Perform elution at 60°C to increase elution efficiency - 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 collected and stored properly - Avoid repeated thawing and freezing
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Increase the evaporation time for Wash Buffer II - Replace drying step with Wash Buffer III (Section 4.2)
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that wash supernatants are efficiently removed - Wash Buffers should be stored and used at RT - Repeat washing step with Wash Buffer II - Replace drying step with Wash Buffer III (Section 4.2)
	Magnetic beads remaining in the eluate	- Place the DNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

5.1 magtivio B.V.

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