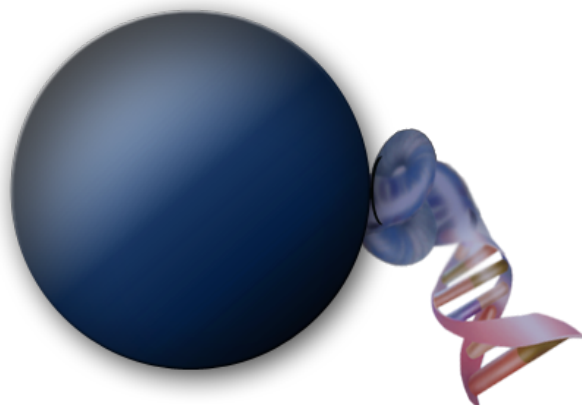


MagSi-DNA Animal

Art.No.

MDKT00150096

MDKT00150960



Product Manual

Revision 6.0 | 03-05-2023



Revision history

Revision	Release date	Remarks
1.0	12/07/2019	Initial release
5.0	08-03-2022	Updated sections 2.2, 3.2, 3.3 and 4.3, layout changes
5.1	08-12-2022	New company style
6.0	03-05-2023	Changes to Chapter 2 Materials (Proteinase K as kit content, equipment/consumables adjusted), Chapter 3 (reagent preparations and lysis recommendations), Chapter 4 (protocol details added)

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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, Lysis Buffer TS or Lysis Buffer VT, 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
Proteinase K		2 x 20 mg	2 x 200 mg
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
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2.2 Reagents, consumables and equipment to be supplied by the user

2.2.1 Reagents (optional)

- Dithiothreitol (DTT) (1 M)
- RNase (10 mg/mL)

2.2.2 Consumables and equipment

Item	Recommended
96 deepwell processing plate	Abgene™ 96 Well 1.2mL Polypropylene DeepWell™ Sample Processing & Storage Plate (Thermo Scientific, REF: AB0564)
96 deepwell extraction plate	Riplate®SW 96, PP, 2ml, (Ritter, REF: 43001-0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0mL, (Thermo Scientific, REF: 278752)
96-well elution plate	Nuclease-free microtiter plate
Magnetic separator	MM-Separator 96 DeepWell, REF: MDMG0013
Resuspension of MagSi-AG IV	Vortex-Genie 2 (Scientific Industries, REF: SI-0236)
Microplate shaker (≥1000 RPM)	Eppendorf ThermoMixer® C (REF: 5382000015)
Heating (Lysis)	Incubator or water bath (≥65°C)
Centrifuge (optional)	Depending on plate type used, recommended >6.000 x g
Tissue homogenizer (optional)	2010 Geno/Grinder® (SPEX SamplePrep)

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

Product	Art. No.	Contents
PurePrep 96 DeepWell Plate	MDPL00200060	60 pieces
PurePrep 96 Elution Plate	MDPL00190060	60 pieces
PurePrep 96 TipComb	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). When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Recommended lysis conditions

MagSi-DNA animal can be used with many different samples. Some samples may not be completely degraded because of their composition, e.g. chitinous materials such as shrimps. However, overnight incubation typically results in sufficient release of nucleic acids into solution. In case highest yields are desired, mechanical disruption may be applied with a commercial homogenizer. Even if no undigested sample debris can be detected after lysis, it is recommended to centrifuge lysates prior to DNA purification.

The following table is intended as a guideline for lysis conditions that can be used for different sample types. Depending on the specific sample type/species, as well as collection and storage conditions, the sample input volume/weight may need to be optimized.

Sample type	Product	Additives	Incubation time
Blood (up to 40 µL)	Lysis Buffer U1 ●	Proteinase K	>15 min
Swab solution (up to 200 µL)	Lysis Buffer U1 ●	Proteinase K	>15 min
Allflex buffer (Ear notch) (≤200 µL)	Lysis Buffer U1 ●	Proteinase K	>15 min
Semen (up to 60 µL)	Lysis Buffer U1 ●	DTT, Proteinase K	>3 h
Hair roots (up to 8 pcs)	Lysis Buffer TS ●	DTT, Proteinase K	>3 h, overnight recommended
Fish fin (up to 40 mg)	Lysis Buffer TS ●	Proteinase K	>3 h
Mammalian tissue (up to 40 mg)	Lysis Buffer TS ●	Proteinase K	>3 h
Insects (e.g. head, thorax)	Lysis Buffer TS ●	Proteinase K	>3 h
Bivalve molluscs (≤30 mg tissue, e.g. gill, mantle or adductor muscle)	Lysis Buffer TS ●	Proteinase K	>1 h
Dried blood spots (up to 3 punches of 3 mm or 1 punch of x 6 mm)	Lysis Buffer VT ●	Proteinase K	>1 h

Please contact technical support for lysis recommendations of samples not listed in the table above.

3.3 Preparation of reagents

3.3.1 Buffers

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




3.3.2 Proteinase K

Reconstitute Proteinase K

- MDKT00230096 (96 preps): add 1.1 mL of diH₂O to each vial of Proteinase K and vortex to dissolve. Store solutions of Proteinase K at -20°C
- MDKT00230960 (10 x 96 preps), add 11 mL of diH₂O to each vial of Proteinase K and vortex to dissolve. Store solutions of Proteinase K at -20°C

3.3.3 Lysis Working Solution

Depending on the sample type (see table in section 3.2), prepare a **Lysis Working Solution** as following:

- Per **400 µL Lysis Buffer U1** , **Lysis Buffer TS**  or **Lysis Buffer VT**  add:
 - 20 µL Proteinase K
 - 20 µL DTT (1 M) (optional, see table in section 3.2)
 - 10 µL RNase A (10 mg/mL) (optional, see section 3.5)
- Prepare a little more Lysis Working Solution than needed due to loss during pipetting (e.g. for 96 extraction prepare solution for 100 extractions).

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 on request.

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 can be optimized.
2. Some samples, such as semen, require DTT to disrupt disulfide bonds of proteins.
3. If samples contain large amounts of RNA, it is recommended to add RNase A (10 mg/mL) to the lysis mixture before incubation.
4. 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 $\geq 50 \mu\text{L}$. 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.
5. Lysis efficiency and DNA yield are highly dependent on the sample type. Different volumes of Lysis Buffer 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 (REF: MDMG0013), allowing for processing in 96 deepwell plates.

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

MagSi-DNA Animal 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 4.2 and 4.3. Software protocol files 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

MagSi-DNA Animal 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 for use

4.1 Manual DNA extraction from animal samples

1. Add samples to a 96 deepwell processing plate of preference for lysis incubation. Optionally, homogenize by mechanical disruption.
2. To each sample, add **400 µL Lysis Working Solution** (see section 3.3) and incubate the samples at 56°C for 1 to 3 hours.

Note: If samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (10 mg/mL) to the lysis mixture.

3. Centrifuge for 15 min (>6.000 x g) to pellet contaminants and cell debris. Transfer **300 µL cleared lysate** to a new 96 deepwell extraction plate.

*Note: Immediately before use, resuspend **MagSi-AG IV** beads by vortexing at maximum speed for 20 sec.*

4. Add **20 µL MagSi-AG IV** and **500 µL Binding Buffer U1** ●. Incubate on a shaker for 5 min at 1000 RPM.
5. Place the sample plate on a magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** ●. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Repeat step 6 one more time for a total of 2 washes with **800 µL Wash Buffer I** ●.
8. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer II** ●. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
9. Dry the beads on air for 10 min to evaporate the ethanol completely.
10. 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.
11. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Transfer the eluted DNA samples to a new microplate for subsequent analysis.
12. *Note: If magnetic particles are accidentally transferred, place the plate on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.*

4.2 Protocol for the PurePrep 96 System

4.2.1 PurePrep 96 software protocol file

Please contact magtivio for the most recent software method files. We provide the corresponding files for direct upload on the PurePrep 96 System. Refer to the PurePrep 96 user manual regarding the upload procedure of the supplied software files to the instrument.

4.2.2 Preparation of processing plates

Plate filling instructions

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	PurePrep 96 Deepwell Plate	Empty, for loading Tip-Comb only	N/A	1
Sample Plate	PurePrep 96 Deepwell Plate	MagSi-AG IV Binding Buffer U1 Lysate	20 µL 500 µL 300 µL	2
Wash Plate 1	PurePrep 96 Deepwell Plate	Wash Buffer I	800 µL	3
Wash Plate 2	PurePrep 96 Deepwell Plate	Wash Buffer I	800 µL	4
Wash Plate 3	PurePrep 96 Deepwell Plate	Wash Buffer II	800 µL	5
Elution Plate	PurePrep 96 Elution Plate	Elution Buffer	150 µL	8

Suitable plates can be purchased at magtivio (see section 2.2.3). 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.

4.2.3 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously (see section 4.2.2) to avoid any misloading during the instrument loading procedure.

Before starting

Depending on the sample material, prepare Lysis Working Solution according to section 3.3.3.

Sample lysis

1. Add samples to a 96 deepwell processing plate of preference for lysis incubation. Optionally, homogenize by mechanical disruption.
2. To each sample, add **400 µL Lysis Working Solution** (see section 3.3) and incubate the samples at 56°C for 1 to 3 hours.

Note: If samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (10 mg/mL) to the lysis mixture.

3. Centrifuge for 15 min (>6.000 x g) to pellet contaminants and cell debris.

DNA purification

*Note: Immediately before use, resuspend **MagSi-AG IV** beads by vortexing at maximum speed for 20 sec.*

4. Prepare the "Sample Plate" for the binding step with **MagSi-AG IV** and **Binding Buffer U1**. Add **20 µL MagSi-AG IV** and **500 µL Binding Buffer U1** ● to the Sample Plate. Transfer **300 µL lysate** to the Sample Plate without disrupting pelleted cell debris.
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 PurePrep 96 Deepwell Plates.
6. Prepare "Wash Plate 3" with **Wash Buffer II**. Add **800 µL Wash Buffer II** ● to each well of the corresponding PurePrep 96 Deepwell Plate.
7. Prepare "Elution Plate" with **Elution Buffer**. Add **150 µL Elution Buffer** ● to each well of the corresponding PurePrep 96 Elution Plate.
8. Switch on the PurePrep 96 System and select the protocol from the user defined protocols.
9. Load all plates to the PurePrep 96 instrument on indicated positions, see section 4.2.2 (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.

10. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
11. At the end of the run remove all plates from the instrument






4.3 Protocol for the KingFisher™ Flex Purification System with 96 Deep-Well Head

4.3.1 KingFisher BindIt software 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.

4.3.2 Preparation of processing plates

Plate filling instructions

Plate name	Plate type	Reagent (Kit component)	Volume
Sample Plate	PurePrep 96 Deepwell Plate	MagSi-AG IV Binding Buffer U1 Lysate 	20 µL 500 µL 300 µL
Wash Plate 1	PurePrep 96 Deepwell Plate	Wash Buffer I 	800 µL
Wash Plate 2	PurePrep 96 Deepwell Plate	Wash Buffer I 	800 µL
Wash Plate 3	PurePrep 96 Deepwell Plate	Wash Buffer II 	800 µL
Elution Plate	PurePrep 96 Elution Plate	Elution Buffer 	150 µL
Tip plate	PurePrep 96 Deepwell Plate	Empty, for loading Tip-Comb only	N/A

Suitable plates can be purchased at magtivio (see section 2.2.3). We strongly recommend using only the plates which are intended to use on the KingFisher Flex™ System. Using unsuitable plates may result in extraction failure or instrument damage.

4.3.3 Detailed instructions

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

Before starting

Depending on the sample material, prepare Lysis Working Solution according to section 3.3.3

Sample lysis

1. Add samples to a 96 deepwell processing plate of preference for lysis incubation. Optionally, homogenize by mechanical disruption.
2. To each sample, add **400 µL Lysis Working Solution** (see section 3.3) and incubate the samples at 56°C for 1 to 3 hours.

Note: If samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (10 mg/mL) to the lysis mixture.

3. Centrifuge for 15 min (>6.000 x g) to pellet contaminants and cell debris.

DNA purification

*Note: Immediately before use, resuspend **MagSi-AG IV** beads by vortexing at maximum speed for 20 sec.*

4. Prepare the "Sample Plate" for the binding step with **MagSi-AG IV** and **Binding Buffer U1**. Add **20 µL MagSi-AG IV** and **500 µL Binding Buffer U1** ● to the Sample Plate. Transfer **300 µL lysate** to the Sample Plate without disrupting pelleted cell debris
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. The purification process starts immediately after loading the sample plate to the instrument.

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.

11. 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
	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
	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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