Steinbrenner Laborsysteme GmbH

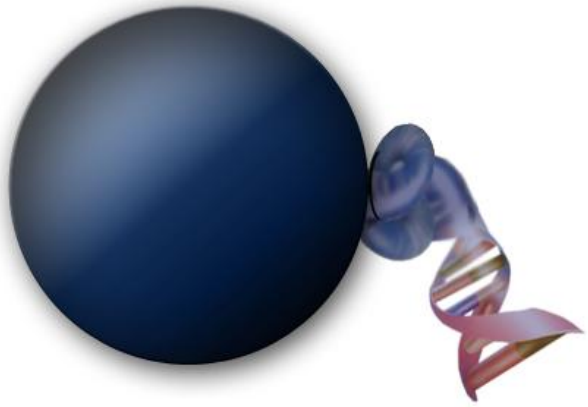
magtivio

MagSi-DNA Animal

Art.No.

MDKT00150096

MDKT00150960



Product Manual

Version 5.1 | 08-12-2022

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

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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
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

- Proteinase K (20 mg/mL), 20 µL per preparation (MDRE00130020 / MDRE00130200, magtivio)
- Dithiothreitol (DTT) (1 M) (optional)
- RNase (10 mg/mL) (optional)

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

Product	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)
Heating	Thermomixer (with adaptor for tubes or plates), incubator or water bath for cell lysis	

2.2.3 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 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

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) Swab solution (up to 200 µL) Tissue preservation buffer (Allflex) (up to 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) Mammalian tissues (up to 40 mg)	Lysis Buffer TS 🔵	Proteinase K	>3 h
Dried blood spots (up to 3 punches of 3 mm or 1 punch of x 6 mm)	Lysis Buffer VT 🟤	Proteinase K	>1h

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.
- Depending on the sample type, prepare a lysis Working Solution as following:
  - Per **400 µL Lysis Buffer U1** 🔴, **Lysis Buffer TS** 🔵 or **Lysis Buffer VT** 🟤 (see table in section 3.2) add:
    - 20 µL Proteinase K (20 mg/mL)
    - 20 µL DTT (1 M) (optional, see table in section 3.2)
    - 10 µL RNase A (10 mg/mL) (optional, see table in section 3.2)
  - 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 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 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

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 Manual DNA extraction from animal samples

Before starting

- Depending on the sample material, prepare Lysis Working Solution according to section 3.3
- Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds

1. 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).

4.2 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	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	Lysate Binding Buffer U1 🟢 MagSi-AG IV	300 µL 500 µL 20 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	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Elution Buffer 🟠	150 µL	8

Suitable plates can be purchased at magtivio (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.

4.2.3 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
- Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds

Sample lysis

1. Transfer the sample to a deepwell microplate or microtube. 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 the "Sample Plate".

DNA purification

3. Prepare the "Sample Plate" for the binding step with **MagSi-AG IV** and **Binding Buffer U1.** To each well of the Sample Plate already containing 300 µL lysate, dispense **20 µL MagSi-AG IV** magnetic beads and **500 µL Binding Buffer U1** 🟢.
4. 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.
5. Prepare "Wash Plate 3" with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
6. Prepare "Elution Plate" with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
7. Switch on the PurePrep 96 System and select the protocol from the user defined protocols
8. 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.
9. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
10. At the end of the run remove all plates from the instrument

4.3 Protocol for the KingFisher Flex™ Magnetic Particle Processor

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	2 ml Deepwell Plate with square wells for KingFisher™/PurePrep 96	Lysate Binding Buffer U1 🟢 MagSi-AG IV	300 µL 500 µL 20 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	2 ml Deepwell Plate with square wells 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

Suitable plates can be purchased at magtivio (see section 2.2). 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
- Immediately before use, resuspend MagSi-AG IV by vortexing for 20 seconds

Sample lysis

1. Transfer the sample to a deepwell microplate or microtube. 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 the "Sample Plate".

DNA purification

3. Prepare the "Sample Plate" for the binding step with **MagSi-AG IV** and **Binding Buffer U1.** To each well of the Sample Plate already containing 300 µL lysate, dispense **20 µL MagSi-AG IV** magnetic beads and **500 µL Binding Buffer U1** 🟢**.**
4. 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.
5. Prepare "Wash Plate 3" with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
6. Prepare "Elution Plate" with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
7. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols
8. Start the protocol.
9. 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.
10. 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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