Steinbrenner Laborsysteme GmbH

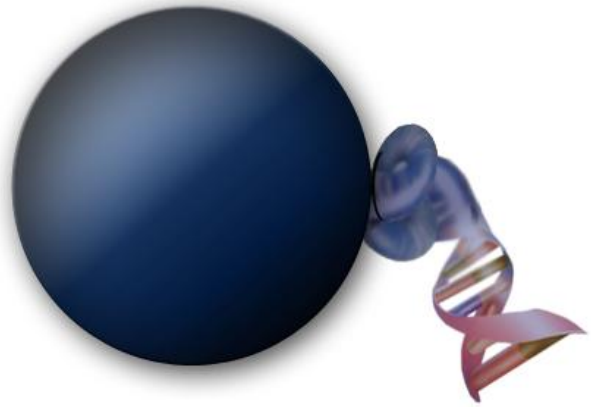
magtivio

MagSi-DNA FFPE

Art.No.

MDKT00240096

MDKT00240960



Product Manual

Version 1.1 | 15/11/2022

Revision history		
Revision	**Release date**	**Remarks**
1.0	15-11-2022	Initial release
1.1	07/12/2022	New company style

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1. General Information

1.1 Intended Use

MagSi-DNA FFPE 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 formalin-fixed, paraffin-embedded (FFPE) mammalian tissue samples or eukaryotic cell sections. Processing time for the preparation of 96 samples is about 40 minutes plus an additional paraffin removal step and pre-lysis incubations of 3 h or overnight for tissue samples. 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 FFPE is suitable for use with scrolls of FFPE tissue or eukaryotic cells.

MagSi-DNA FP11 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. Depending on the sample materials RNA may be co-purified. If required, RNase treatment has to be integrated in the purification protocol. RNase is not included in the kit.

1.2 Kit specifications

The kit provides reagents for extraction of DNA from up to four FFPE scrolls of up to 20 µm thickness. Depending on the size and morphology of the embedded tissue and the size of the FFPE scrolls the maximum tissue weight can be up to 25 mg. The maximum size of the FFPE scrolls should not exceed 500 mm2 per individual scroll. The DNA yield depends on the tissue or cell sample and can be highly variable. Typically an A260/A280 ratio of >1.7 and A260/A230 ratio of >1.5, with typical concentrations between 20 ng/µL and 50 ng/µL can be obtained.

The DNA obtained can be stored at 2-8°C. For long-term storage of purified DNA, 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

Paraffin is removed by dissolving in xylene or by melting at high temperature and transferring the aqueous phase. Next, the samples are lysed under denaturing conditions by adding Lysis Buffer FP and Proteinase K at 56°C and additionally at 90°C to revert cross-links. After the lysis incubations, remaining debris is removed by centrifugation. MagSi-FP11 beads are added to the cleared supernatant and 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 MDKT00240096	10 x 96 preps MDKT00240960
Lysis Buffer FP	25 mL	250 mL
Binding Buffer U1	40 mL	400 mL
Proteinase K	4 x 20 mg (for 1.1 mL working solution, each tube)	4 x 200 mg (for 11 mL working solution, each tube)
MagSi-FP11	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

2.2 Reagents, consumables and equipment to be supplied by the user

2.2.1 Reagents

- Xylene and 96 % ethanol (non-denatured) for paraffin removal
- molecular biology grade (nuclease free) water to reconstitute Proteinase K
- If RNA-free DNA is required: RNase A solution 20 mg/mL, 20 µL per sample

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

Protocol	Manual use	Automated use
Deparaffinization and sample lysis	Recommended: conical 1.5 ml safe lock tubes or screw cap tubes for lysis	
Processing 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
Deparaffinization / Lysis / Elution / mixing	Heater shaker for tubes / tube Vortexer	Heater microplate shaker (min. 1000 RPM)

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 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 each vial of Proteinase K:
- MDKT00240096 (96 preps), add **1.1 mL** of **diH2O** to **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K at -20°C
- MDKT00240960 (10x96 preps), add **11 mL** of **diH2O** 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-FP11 beads by vortexing for 20 seconds. If preferred, MagSi-FP11 beads can be premixed with Binding Buffer U1 for simultaneous addition to samples. The mixture must be 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-FP11	20 µL
Total	420 µL

- Samples should be thoroughly mixed before aliquotation

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 www.magtivio.com under each magtivio kit and kit component.

Infectious potential of liquid waste left over after using the MagSi-DNA FFPE kit 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. Elution at RT is possible but may decrease yields.
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. The kit is intended to be used for up to four FFPE scrolls of up to 20 µm thickness each. Exceeding the sample amount may result in lower DNA yield due to incomplete bead separations out of viscous sample lysates or due to low purity.
8. It may occur that a small amount of beads is accidentality transferred 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 FFPE 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 FFPE 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 4.4 . Software protocol files are available on request.

3.6 Shaker settings

The speed settings for the microplate shaker in the following protocols that follow were defined for 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 FFPE 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 samples mentioned The kit is not validated for isolating DNA from for instance stool, 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. Depending on applicable regulations, 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 genomic applications, such as restriction digestion, qPCR, 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 Deparaffinization

When preparing fresh FFPE scrolls from blocks, cut away access of paraffin from the block. Discard first three scrolls before collecting scrolls for the subsequent purification protocol.

1. Transfer the FFPE scrolls into suitable microtubes. It is recommended to use conical safe lock or screw cap tubes.
2. Add **950 µL Xylene** to each sample tube. Make sure that the FFPE curls are submerged completely. Incubate the samples for 3 min at 55°C to melt the paraffin.
3. Centrifuge the sample tubes for 2 min (>4,000 g) at room temperature to collect the tissue samples on the bottom of the tubes.
4. Using a 1 mL pipette, aspirate and discard as much xylene as possible without aspirating the pelleted tissue or cell samples. If the samples are not visible it is recommended to leave about 50 µL xylene behind inside the sample tubes.
5. Add **1 mL of 96 % ethanol** to each sample tube and mix by vortexing.
6. Centrifuge the sample tubes for 2 min (>4,000 g) at room temperature to collect the tissue samples on the bottom of the tubes.
7. Using a 1 mL pipette, aspirate and discard as much ethanol as possible without aspirating the pelleted tissue or cell samples. If the samples are not visible leave about 50 µL ethanol behind inside the sample tubes.
8. Briefly centrifuge the sample tubes and remove any remaining ethanol with a fine pipette.
9. Air-dry the pelleted samples for 15 min at room temperature or 3-10 min at 60°C until no large ethanol droplets are visible. Excess of remaining ethanol may inhibit the proteinase K in the subsequent lysis step.
10. Continue with sample lysis (section 4.2, step 1).

User note:

A single FFPE scroll can be subjected to the lysis incubation directly without prior paraffin removal step described above in section 4.1. This is only recommended for processing a single FFPE scroll of up to 20 µm thickness and up to 500 mm2 size. The direct lysis procedure avoids the use of reagents for paraffin removal like xylene or mineral oil, but may result in slightly lower purity of the DNA and remaining traces of magnetic beads in the DNA eluate. The paraffin will melt during the lysis incubation at 56°C. After the heat incubations (lysis at 56°C and decrosslinking at 90°C) the paraffin will solidify again upon cooling down the sample to room temperature. The solidified paraffin will form a thin layer on top of the lysate that needs to be avoided during transfer of the lysate in step 3 of section 4.2.

4.2 Manual DNA extraction

Before starting

- Deparaffinize the samples as described 4.1
- It is recommended to use safe lock or screw cap tubes to for the heat incubations to avoid evaporation.
- Check if Proteinase K was prepared according to section 3.2 .
- Before use of MagSi-FP11 beads in step 5 vortex the magnetic beads thoroughly into a homogeneous suspension.

1. Add **225 µL Lysis Buffer FP** and **40 µL Proteinase K** to the deparaffinized sample. Spin down briefly. Incubate tissue samples for at least 2 h or overnight with moderate shaking at 56°C. The sample should be disintegrated after the lysis incubation. Make sure that the tissue samples are submerged into the lysis buffer during lysis incubation.
2. Incubate for 1 h at 90°C. Cool down to room temperature. The additional incubation at 90°C reverts crosslinks and may increase the yield in the DNA extraction process. Spin down briefly after incubation.

   If RNA free DNA is required: Add 20 µL of a 20 mg/mL RNase A solution (not supplied with the kit) after the lysis incubation. Incubate with shaking for 10 min at room temperature.
3. Centrifuge the lysed sample for 5 min at full speed to collect any unlysed material to the bottom of the container used for processing. Transfer 200 µL of the lysate supernatant to a new container. Avoid transfer of undigested sample material or debris.
4. Add **400 µL Binding Buffer U1** and **20 µL MagSi-FP11.** Incubate on a shaker for 5 min at 1000 RPM.
5. Place the samples on the 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** 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.
7. Repeat step 6 one more time with **800 µL Wash Buffer I** and once with **800 µL Wash Buffer II**
8. Dry the beads on air for **5 min** to evaporate the ethanol completely.
9. Remove the sample plate from the magnetic separator and add **100 µL Elution Buffer** . Incubate at 72°C on a shaker for 5 min at 1000 RPM.

10. 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.*

4.3 Protocol for the PurePrep 96

4.3.1 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.

4.3.2 Sample lysis (offline)

1. Deparaffinize, lyse and decrosslink the samples as described in section 4.1
2. Transfer 200 µL of the cleared lysate to a 2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96. Avoid transfer of undigested sample material or debris.

4.3.3 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 MagSi-FP11 beads Binding Buffer U1	200 µL 20 µL 400 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 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.

4.3.4 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 the “Sample Plate” for the binding step with **MagSi-FP11** and **Binding Buffer U1.** To each well of the Sample Plate already containing 200 µL lysate, dispense **20 µL MagSi-FP11** magnetic beads and **400 µL Binding Buffer U1** 🟢**.**
2. 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.
3. Prepare “Wash Plate 3” with **Wash Buffer II**. Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
4. Prepare “Elution Plate” with **Elution Buffer.** Add **100 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
5. Switch on the PurePrep 96 System and select the protocol from the user defined protocols
6. 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.
7. Press on the Tab “Run Prog.”, select the shortcut icon for the protocol and press Run to start the protocol
8. At the end of the run remove all plates from the instrument

4.4 Protocol for the KingFisher Flex™

4.4.1 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.

4.4.2 Sample lysis (offline)

1. Deparaffinize, lyse and decrosslink the samples as described in section 4.1
2. Transfer 200 µL of the cleared lysate to a 2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96. Avoid transfer of undigested sample material or debris.

4.4.3 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 MagSi-FP11 beads Binding Buffer U1	200 µL 20 µL 400 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	100 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 PurePrep 96 System. Using unsuitable 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 the “Sample Plate” for the binding step with **MagSi-FP11** and **Binding Buffer U1.** To each well of the Sample Plate already containing 200 µL lysate, dispense **20 µL MagSi-FP11** magnetic beads and **400 µL Binding Buffer U1** 🟢.
2. 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.
3. Prepare “Wash Plate 3” with **Wash Buffer II**. Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deep-well plate.
4. Prepare “Elution Plate” with **Elution Buffer.** Add **100 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
5. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols
6. Start the protocol.
7. 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.
8. 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	Too much sample material	- Try lower sample input amount, avoid high sample amounts in combination with low elution buffer volumes.
	Incomplete lysis	- Increase incubation time for lysis. - Check the required temperature for Proteinase K digestion (56°C). - Make sure that the sample is submerged in lysis buffer. If possible use heater shaker for optimal lysis. - Poor sample quality.
	No decrosslinking step	- Omitting the decrosslinking step will reduce the DNA yield significantly.
	Inefficient binding to the magnetic particles	- Make sure Lysis Buffer FP 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. - Make sure that the magnetic beads are dispensed. completely in the elution buffer.
Degraded or sheared DNA	Storage and processing of the sample material	- Sample storage before formalin fixation and the process of formalin fixation, paraffin embedding, paraffin removal causes degradation of the sample DNA.
Purified DNA samples are turbid	Sample lysates contains solid particulates	- Centrifuge briefly after lysis incubation and transfer samples to a new container.
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Remove remaining traces from Wash Buffer II completely. 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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