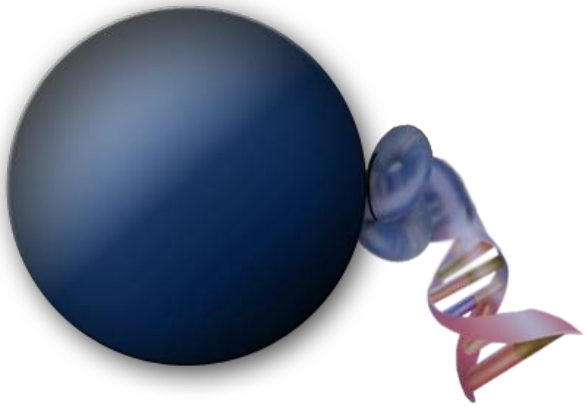
magtivio

MagSi-DNA Soil

REF:
MDKT00280096



Product Manual

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Revision History		
Revision Number	Release Date	Changes
1.0	08-01-2025	Initial release

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1. General Information

1.1. Intended Use

MagSi-DNA Soil 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 microbial DNA from soil samples. Processing time for the preparation of 96 samples is about 90 minutes. The kit requires no phenol/chloroform extraction or alcohol precipitation and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The obtained DNA can be used directly for downstream applications such as PCR, or any kind of enzymatic reaction.

1.2. Specifications

MagSi-DNA Soil is suitable for use with up to 250 mg soil sample per extraction. DNA yield is highly dependent on the sample and its storage condition. Typically, DNA concentrations of 10-50 ng/µL with a A260/A280 ratio of >1.7 and A260/A230 ratio of >1.5 can be obtained.

The DNA 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 multiple freeze-thaw cycles.

1.3. Principle of operation

Soil samples are mechanically disrupted in Homogenization Tubes SL and incubated at 70°C for lysis under denaturing conditions by adding Lysis Buffer SL and Proteinase K. After the lysis incubation, remaining debris are removed by centrifugation and cleared lysates are transferred to a new container. Precipitation Buffer SL is added for precipitation of inhibitors at 2-8°C. The precipitates are removed by centrifugation and the supernatants are transferred to a new container. MagSi-SL beads are added and binding conditions are adjusted by addition of Binding Buffer SL so that DNA binds to the magnetic beads. After magnetic separation and discard of the supernatant, the beads are washed four times to remove contaminants and salts. Finally, purified DNA is eluted and can directly be used for downstream applications.

2. Materials

2.1. Contents

Component	96 Preps MDKT280096
Homogenization Tubes SL	96 pcs
Lysis Buffer SL	55 mL
Proteinase K	2x 1 mL
Precipitation Buffer SL	10 mL
MagSi-SL	2 mL
Binding Buffer SL	30 mL
Wash Buffer SL1	2 x 80 mL
Wash Buffer SL2	80 mL
Wash Buffer SL3	80 mL
Elution Buffer SL	20 mL

2.2. Materials to be supplied by the user

2.2.1. Equipment

Item	Recommended
Sample homogenizer	2010 Geno/Grinder® (SPEX SamplePrep)
Heating (Lysis)	Incubator or water bath (≥65°C)
Centrifuge for 2 mL microtubes	10.000 – 20.000 x g
Magnetic separator	MM-Separator 96 DeepWell, REF: MDMG0013
Microplate shaker (≥1000 RPM)	Eppendorf ThermoMixer® C (REF: 5382000015)

2.2.2. Consumables

Item	Recommended
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
Pipettes and tips	Nuclease-free, 20-200-1000 µL

2.2.3. Reagents

- If RNA-free DNA is required: 20 µL RNase A (20 mg/mL) per sample

2.2.4. Consumables for use on PurePrep 96 System or KingFisher Flex

Product	Art. No.	Contents
PurePrep 96 DeepWell Plate	MDPL00200060	60 pieces
PurePrep 96 Elution Plate	MDPL00190060	60 pieces
PurePrep 96 TipComb	MDPL00210060	60 pieces
KingFisher 96 deep-well plate	ThermoFisher Scientific, REF: 95040450	
KingFisher 96 tip comb for deep-well magnets	ThermoFisher Scientific, REF: 97002534	
KingFisher 96 microplate	ThermoFisher Scientific, REF: 97002540	

3. Kit usage

3.1. Storage Conditions

All components of the kit should 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. 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 can be found in the safety data sheets (SDS), available at www.magtivio.com under each magtivio kit and kit component.

Infectious potential of leftover liquid waste after using the MagSi-DNA Soil 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.

3.3. Preparation of reagents

Briefly spin down the Homogenization Tubes to bring down the grinding beads before opening.

3.4. Homogenization and lysis options

High-powered bead-beating is recommended for homogenization. The following instruments and settings can be used:

Instrument	Conditions
Vortex Genie 2	10 minutes at full speed
Retsch Mixer Mill MM 400	2 minutes at highest frequency (30 Hz)
Bertin Precellys Evolution	4 x 1 minute at 9,000 RPM with 2 minutes rest
Geno/Grinder 2010	5 x 1 minute at 1,500 RPM with 15 seconds rest

3.5. Magnetic Separation systems

MagSi-DNA Soil 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 Soil 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 and use this setting for mixing steps in the extraction procedure.

3.7. Considerations

1. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter tips.
2. Depending on the sample material RNA may be co-purified. If required, Rnase treatment can be integrated in the purification protocol. RNase is not included in the kit.
3. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.
4. Do not combine components of different kits unless the lot numbers are identical.
5. Process only as many samples in parallel as the magnetic separator allows.
6. The elution can be done in smaller volumes of Elution Buffer SL. 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.
7. The kit is intended to be used for up to 250 mg soil. Exceeding the sample amount may result in sample and inhibitory compound carry-over to the final eluate. The finally obtained DNA eluate should be clear and not colored.
8. It may occur that a small amount of beads is accidentally 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.

3.8. Product use limitations

MagSi-DNA Soil 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 soil. The kit is 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 kit have not been validated for any specific application. 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.

4. Protocol for use

4.1. Sample homogenization and lysis

*Note: before opening, briefly spin down the grinding beads in the **Homogenization Tubes SL**.*

1. Add up to 250 mg soil sample per **Homogenization Tube SL**.
2. Add **550 µL Lysis Buffer SL** and **20 µL Proteinase K** to each sample and close the tubes carefully.
3. Disrupt the sample by bead beating in a Geno/Grinder for 5 x 1 min at 1500 RPM. Settings for different homogenization devices are provided in section 3.4. Alternatively, use another instrument and homogenize according to manufacturer's recommendations.
4. Incubate the suspension for 10 min at 70 °C.
5. Centrifuge the sample tubes for 10 min at 10.000 – 20.000 x g to collect debris and remaining unlysed sample material to the bottom of the tubes.
6. Transfer **300 µL supernatant** to new 2 mL tubes. Avoid transfer of debris or unlysed sample material.
7. Add **100 µL Precipitation Buffer SL**, vortex and incubate 10 min at 2-8 °C.
8. Centrifuge the samples for 10 min at 10.000 – 20.000 x g to collect precipitates to the bottom of the tubes.
9. Transfer **300 µL supernatant** to 96 deepwell plate for manual processing or a PurePrep 96 Deepwell Plate. Proceed with section 4.2, 4.3 or 4.4.

4.2. Manual DNA purification

*Note: Immediately before use, resuspend **MagSi-SL** beads by vortexing at maximum speed for 20 seconds*

1. Add **20 µL MagSi-SL** beads and **300 µL Binding Buffer SL** to the cleared lysate. Incubate on a plate shaker for 5 min at 1000 RPM.
2. Place the samples on the **MM-Separator 96 Deepwell** and wait for 1 min to collect the beads. Remove supernatants.
3. Remove the sample plate from the **MM-Separator 96 Deepwell** and add **800 µL Wash Buffer SL1** to the tubes. Incubate on a shaker for 1 min at 1000 RPM. Place the tubes in the **MM-Separator 96 Deepwell** and wait for 1 min to collect the beads. Remove the supernatants.
4. Repeat step 3 one more time with **800 µL Wash Buffer SL1** and once with **800 µL Wash Buffer SL2**.
5. Leave the sample plate on the **MM-Separator 96 Deepwell** (!) and slowly add **800 µL Wash Buffer SL3** without resuspension of the beads. Within 30 seconds after addition remove the supernatants again, avoiding disruption of the beads pellet without resuspension of the samples.
6. Remove the sample plate from the magnetic separator and add **150 µL Elution Buffer SL**. Incubate for 5 min at 1000 RPM and 72 °C.
7. Place the tubes on the **MM-Separator 96 Deepwell** and wait for 1 minute to collect the beads. Transfer the eluates to a 96-well elution plate or different container of preference. The DNA in the eluate is now ready for downstream use.
    - 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. DNA Purification on the PurePrep 96 System

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.

4.3.1. 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	Lysate MagSi-SL Binding Buffer SL	300 µL 20 µL 300 µL	2
Wash Plate 1	PurePrep 96 Deepwell Plate	Wash Buffer SL1	800 µL	3
Wash Plate 2	PurePrep 96 Deepwell Plate	Wash Buffer SL1	800 µL	4
Wash Plate 3	PurePrep 96 Deepwell Plate	Wash Buffer SL2	800 µL	5
Wash Plate 4	PurePrep 96 Deepwell Plate	Wash Buffer SL3	800 µL	6
Elution Plate	PurePrep 96 Deepwell Plate	Elution Buffer SL	150 µL	8

Suitable plates can be purchased at magtivio (see section 2.2.4). 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.

4.3.2. PurePrep 96 instructions

1. Switch on the PurePrep 96 System and select the protocol from the user defined protocols.
2. Load all plates to the PurePrep 96 instrument on indicated positions as listed in right-most column in the table above. Use the clockwise/counter clockwise buttons on the instrument to rotate the turntable to the indicated positions.
3. 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.
4. Press on the Tab “Run Prog.”, select the shortcut icon for the protocol and press Run to start the protocol
5. At the end of the run, remove all plates from the instrument.

4.4. DNA purification with the KingFisher Flex™

4.4.1. KingFisher BindIt software protocols

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. 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

Plate filling instructions:

Plate name	Plate type	Reagent (Kit component)	Volume
Sample Plate	KingFisher 96 deep-well plate	Lysate MagSi-SL Binding Buffer SL	300 µL 20 µL 300 µL
Wash Plate 1	KingFisher 96 deep-well plate	Wash Buffer SL1	800 µL
Wash Plate 2	KingFisher 96 deep-well plate	Wash Buffer SL1	800 µL
Wash Plate 3	KingFisher 96 deep-well plate	Wash Buffer SL2	800 µL
Wash Plate 4	KingFisher 96 deep-well plate	Wash Buffer SL3	800 µL
Elution Plate	KingFisher 96 deep-well plate or KingFisher 96 microplate	Elution Buffer SL	150 µL
TipLoad Plate	KingFisher 96 deep-well plate	Empty, for loading Tip-Comb only	N/A

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.

4.4.3. Kingfisher Flex™ instructions

1. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols. Start the protocol.
2. Load the plates to the instrument, following the instructions on the instrument display. 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. The purification process starts immediately after loading the sample plate to the instrument.
3. At the end of the method, remove all plates from the instrument. Follow the instructions on the instrument display.