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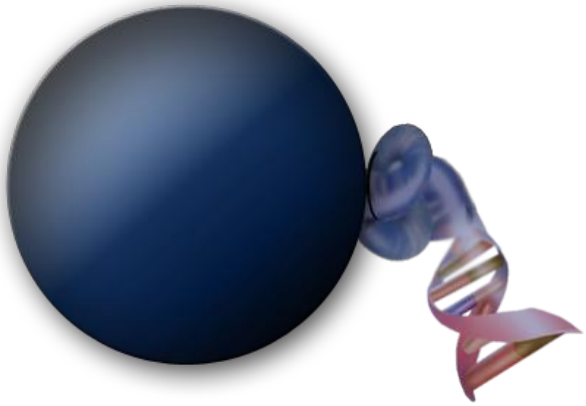
MagSi-DNA Stool

Art.No.

MDKT00230096 / MDKT00230960

MDKT0023B096 / MDKT0023B960



Product Manual

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Revision history		
Revision	**Release date**	**Remarks**
1.0	03-03-2023	Initial release

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1. General Information

1.1 Intended Use

MagSi-DNA Stool 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 microbiome DNA from fresh, frozen or stabilized human stool samples. Processing time for the preparation of 96 samples is about 60 minutes. 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.

1.2 Kit specifications

MagSi-DNA Stool is suitable for use of up to 200 mg fresh or frozen stool or 200 µL preserved stool samples. DNA yield depends on the sample, its storage condition and can be variable. Typically, DNA concentrations of up to 20-50 ng/µL with a A260/A280 ratio of >1.7 and A260/A230 ratio of >1.5 can be obtained.

The kit has been tested for use with fresh or frozen stool, stool collected in OMNIgene®·GUT (DNA Genotek, REF: OM-200) or preserved in DNA/RNA Shield (Zymo Research, REF: R1100-50).

The DNA obtained 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 Basic principle

Stool samples are resuspended and lysed under denaturing conditions by adding Lysis Buffer SLB and Proteinase K at 70°C. For difficult-to-lyse bacteria mechanical homogenization is recommended before incubation. Alternatively incubation step at 90°C can be performed. After the lysis incubations, remaining debris are removed by centrifugation and cleared lysates are transferred to a new container. MagSi-ST10 beads are added and binding 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.

1.4 Lysis and homogenization

MagSi-DNA Stool does not require homogenization. However, for more demanding applications mechanical disruption with GP Lysis Tubes is recommended. If the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a bead beater is desired, high-powered bead beating may be used, for instance with the Geno/Grinder® (SPEX Sample Prep).

2. Materials

2.1 Kit Contents

	96 preps MDKT00230096	10 x 96 preps MDKT00230960	96 preps MDKT0023B096	10 x 96 preps MDKT0023B960
Lysis Buffer SLB	110 mL	1.1 L	110 mL	1.1 L
Binding Buffer U1	40 mL	400 mL	40 mL	400 mL
Proteinase K	4 x 20 mg (for 4 x 1.1 mL working solution,)	4 x 200 mg (for 4 x 11 mL working solution)	4 x 20 mg (for 4 x 1.1 mL working solution)	4 x 200 mg (for 4 x 11 mL working solution)
MagSi-ST10	2 mL	20 mL	2 mL	20 mL
Wash Buffer I	2 x 80 mL	2 x 800 mL	2 x 80 mL	2 x 800 mL
Wash Buffer II	80 mL	800 mL	80 mL	800 mL
Elution Buffer	20 mL	200 mL	20 mL	200 mL
GP Lysis Tubes	*Not included*	*Not included*	100 pcs	10 x 100 pcs
Manual	1	1	1	1

2.2 Optional accessories available separately

Product	Art. No.	Contents
MM-Separator M12 + 12 P	MDMG0001	1 unit
GP Lysis Tubes	MDPL00330100	100 pieces

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

2.3.1 Reagents

- 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

2.3.2 Equipment and consumables

- Optional: Vortex Mixer with adaptor for 2 mL tubes or high speed bead beater
- Optional: GP Lysis Tubes (see section 2.2)
- Centrifuge
- Magnetic separator, recommended: MM-Separator M12 + 12 P
- Thermoshaker for 2 mL tubes, or incubator capable of 70°C (or 90°C for lysis without homogenization)
- 2 mL microtubes
- Pipettes and tips

2.3.3 Consumables for the PurePrep 96 System

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 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 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.

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

- Reconstitute each vial of Proteinase K:
  - MDKT00230096 / MDKT0023B096 (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 / MDKT0023B0960 (10 x 96 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-ST10 beads by vortexing for 20 seconds. If preferred, MagSi-ST10 beads can be premixed with Binding Buffer U1 for simultaneous addition to samples. The mixture should 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-ST10	20 µL
Total	420 µL

- Samples should be thoroughly mixed before aliquotation

3.4 Bead beating and lysis options

MagSi-DNA Stool can be used without mechanical disruption for less demanding applications. If the microorganism of interest requires bead beating (e,g, Lactobaccilus), bead beating with GP Lysis Tubes is recommended. Most microorganisms can be treated successfully by bead beating horizontally on a Vortex Mixer, but if high-powered bead-beating is desired a mixer mill such as the SPEX SamplePrep Geno/Grinder can be used. We recommend the following settings for homogenizaton:

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 minutes 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 Stool has been designed for manual use on the MM-Separator M12 + 12 P (Art.No. MDMG0001) allowing simultaneous processing of up to 12 samples in 2 mL microcentrifuge tubes.

For (automated) processing in 96 deepwell plates, the MM-Separator 96 DeepWell (Art.No. MDMG0013) is recommended.

For use with other magnetic separators, please contact the technical support at info@msbio.com

MagSi-DNA Stool is compatible with PurePrep 16, 32, and 96 systems 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.

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. Elution can be performed at room temperature, but may decrease yields slightly.
4. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.
5. Do not combine components of different kits unless the lot numbers are identical.
6. Process only as many samples in parallel as the magnetic separator allows.
7. 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.
8. The Elution Buffer does not contain EDTA (the end user may wish to use other elution buffers containing EDTA, or Tris and EDTA, though).
9. The kit is intended to be used for up to 200 mg of solid stool. 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 coloured. Any colouration indicates and overloading / using to much sample material of the purification process.
10. 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.

3.8 Product use limitations

MagSi-DNA Stool 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 human stool samples. 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 kits have not been validated for any specific application. Depending on applicable regulations, 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.

4. Protocols

4.1 Sample homogenization and lysis

Before starting:

- *Check if Proteinase K was prepared according to section 3.3*
- *If RNA-free DNA is required: Add 20 µL of a 20 mg/mL RNase A solution (not supplied with the kit) to the lysed sample. Incubate with shaking for 10 min at room temperature.*

4.1.1 Enzymatic / heat lysis:

1. Transfer up to 200 mg or 200 µL stool sample into suitable microtubes (e.g. screw cap tubes or safe lock microtubes).
2. Add **40 µL of reconstituted Proteinase K** and **1 mL Lysis Buffer SLB** ● to each sample. Vortex 30-60 sec to obtain a homogenous sample suspension.
3. For sample lysis, incubate the suspension for 10 min at 70°C with shaking at 1000 RPM. For difficult-to-lyse bacteria, Incubate the suspension for an additional 10 min at 90°C.
4. Centrifuge the sample tubes for 10 min at maximum speed at room temperature to collect debris and remaining unlysed sample material to the bottom of the tubes. The obtained sample lysate should be clear after centrifugation.
5. Aspirate 200 µL from the cleared lysates and transfer to new 2 mL tubes or a PurePrep 96 Deepwell Plate. Avoid transfer of debris or unlysed sample material. Proceed at section 4.2 , 4.3, or 4.4.

4.1.2 Lysis with bead beating in GP Lysis Tubes

1. Briefly spin down the grinding beads in the **GP Lysis Tubes** before opening. Transfer up to 200 mg or 200 µL stool sample into the GP Lysis Tubes.
2. Add **40 µL of reconstituted Proteinase K** and **1 mL Lysis Buffer SLB** ● to each sample and close the tubes carefully.
3. Secure the GP Lysis Tubes horizontally on a Vortex Adapter. Vortex the samples for 10-15 min with highest speed setting. Alternatively use a high speed mixer mill and homogenize according to manufacturer's recommendations.
4. Incubate the suspension for 10 min at 70°C with shaking at 1000 RPM.
5. Centrifuge the sample tubes for 5 min at maximum speed at room temperature to collect debris and remaining unlysed sample material to the bottom of the tubes. The obtained sample lysate should be clear after centrifugation.
6. Aspirate 200 µL from the cleared lysates and transfer to new 2 mL tubes or a PurePrep 96 Deepwell Plate. Avoid transfer of debris or unlysed sample material. Proceed at section 4.2 , 4.3, or 4.4.

4.2 Manual DNA purification

1. Add **400 µL Binding Buffer U1** 🟢 and **20 µL MagSi-ST10 beads** to the cleared lysate. Incubate on a shaker for 5 min at 1000 RPM.
2. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
3. 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.
4. Repeat step 3 one more time with **800 µL Wash Buffer I** 🔵 and one time with **800 µL Wash Buffer II** 🟡.
5. Dry the beads on air for **5 min** to evaporate the ethanol completely.
6. Remove the sample plate from the magnetic separator and add **200 µL Elution Buffer** 🟠. Incubate at 72°C on a shaker for 5 min at 1000 RPM.
7. Place the tubes in a magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes.
8. 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 DNA purification with the PurePrep 96 System

Please contact AMSBIO 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-ST10 beads Binding Buffer U1	200 µL 20 µL 400 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	100 µL	8

Suitable plates can be purchased at AMSBIO (see section 2.3.3).

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 protocol

Please contact AMSBIO 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	PurePrep 96 Deepwell Plate	Lysate MagSi-ST10 beads Binding Buffer U1	200 µL 20 µL 400 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	100 µL
Tip plate	PurePrep 96 Deepwell Plate	Empty, for loading Tip-Comb only	N/A

Suitable plates can be purchased at AMSBIO (see section 2.3.3).

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.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield or low DNA purity	To much sample material	- Try lower sample input amount, avoid high sample amounts in combination with low elution buffer volumes.
	Incomplete lysis	- Suspend sample in Lysis Buffer SLB to a homogeneous suspension. Vortex or use a shaker to achieve homogeneous sample suspension. - Check the required temperature for Proteinase K digestion (70°C). Consider second incubation at 90°C to achieve higher lysis efficacy. - Use a heater shaker for optimal lysis. - Poor sample quality.
	Inefficient binding to the magnetic particles	- Make sure Lysis Buffer SLB 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 completely dispensed in the elution buffer.
Degraded or sheared DNA	Storage and processing of the sample material	- Sample storage before DNA extraction: Freeze collected samples immediately or use dedicated stabilisation tubes. Avoid repeated freeze/thaw cycles of the sample.
Purified DNA samples are turbid or coloured	Sample lysates contains solid particulates	- Centrifuge briefly after lysis incubation and transfer samples to a new container. - Use less sample material for DNA extraction.
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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