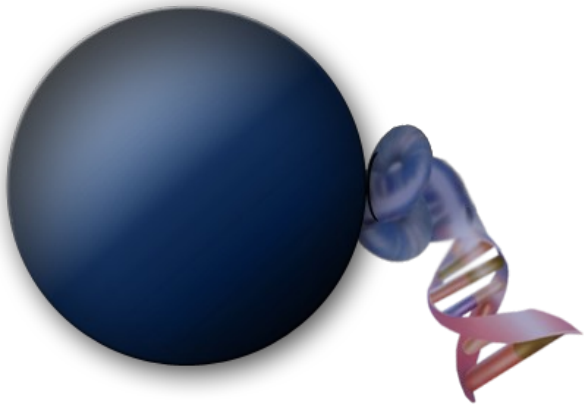
Steinbrenner Laborsysteme GmbH

magtivio

MagSi-cfDNA

Art.No.

MDKT00220096



Product Manual

Version 3.0 | 10/05/2023

Steinbrenner Laborsysteme GmbH

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Revision history		
Revision	**Release date**	**Remarks**
1.0	25-11-2021	Initial release
2.0	21-02-2022	Text corrections in section 4.3
2.1	07-12-2022	New company style
3.0	10-05-2023	Addition of urine protocol, text corrections

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Table of Contents

1. General Information

1.1 Intended Use

MagSi-cfDNA is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

The kit is intended for isolation of circulating cell-free DNA from human plasma, serum and urine samples. Processing time for the preparation of 24 samples is about 60 minutes. Following the lysis incubation at 56°C (only for plasma and serum samples), all other steps of the procedure are processed at room temperature. 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 cfDNA can be used directly for downstream applications such as PCR, or any kind of enzymatic reaction.

MagSi-cfDNA is optimized for use on the PurePrep 24 System and is suitable for use with fresh or frozen plasma or serum and urine samples. Plasma samples must be prepared from blood samples collected in a suitable collection tube (e.g. Cell-Free DNA BCT® (Streck), etc.)

1.2 Kit specifications

The kit provides reagents for 96 extractions of cfDNA from 2 mL sample volume, but is scalable for use between 1 and 4 mL sample for plasma and serum and up to 10 mL for urine. Yield of purified cfDNA is highly variable from donor to donor. Typically 0.5 to 4 ng cfDNA can be obtained per 1 mL of human plasma. The obtained cfDNA can be stored at 2-8°C. For long-term use, storage at -20°C is recommended.

1.3 Principle of operation

Samples are lysed under denaturing conditions by adding Lysis Buffer CF and Proteinase K at 56°C (for human plasma and serum samples). After lysis incubation MagSi-CF8 beads are added and binding conditions are adjusted by addition of Binding Buffer CF so that nucleic acids bind to the magnetic beads. After magnetic separation and discard of the supernatant, the beads are washed a total of three times with alcoholic Wash Buffer CF1 and Wash Buffer CF2 to remove contaminants and salts. A drying step makes sure all traces of ethanol are removed. Finally, purified nucleic acids are eluted with low-salt elution buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

Component	96 preps MDKT00220096
Lysis Buffer CF	50 mL
Binding Buffer CF	420 mL
Proteinase K	200 mg (for 11 mL of working solution)
MagSi-CF8	3 mL
Wash Buffer CF1	320 mL
Wash Buffer CF2	2 x 320 mL
Elution Buffer	20 mL
Manual	1

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

2.2.1 Reagents

- diH_2O (to reconstitute Proteinase K)

2.2.2 Consumables/equipment for manual processing

Item	Recommended
Containers for sample processing	50 mL centrifugate tubes and 2 mL microtubes
Magnetic separator for 50 mL centrifugate tubes	MM-Separator 50 P (magtivio, REF: MDMG0015)
Magnetic separator for 2 mL microtubes	MM-Separator M12 + 12 P (magtivio, REF: MDMG0001)
Final sample container	1.5 or 2 mL microtubes, or 96 well microplate
Heater/shaker for lysis and mixing	Eppendorf ThermoMixer® C (REF: 5382000015) with adaptors for 50 mL tubes and 2 mL tubes
Centrifuge to collect liquid to the bottom after incubation with shaking	Eppendorf™ Centrifuge 5430 (REF: 5427000015)

2.2.3 Consumables/equipment for processing on the PurePrep 24 System

Product	REF	Contents
PurePrep 24 System	AS00003	1 unit
PurePrep 24 DeepWell Plate	MDPL00280050	50 pcs / box
PurePrep 24 Tip-Comb + 24 DeepWell Plate	MDPL00290050	50 pcs / box

3. Kit usage

3.1 Storage Conditions

All components of the MagSi-cfDNA 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. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents.

3.2 Preparation of reagents

All components except Proteinase K are ready-to-use. MagSi-CF8 beads should be well resuspended immediately before use. If there is any precipitate present in the buffers, warm up to 25-37°C to dissolve the precipitate.

Reconstitute Proteinase K:

- Add **11 mL diH$_2$O** to **Proteinase K** and vortex to dissolve. Store working solutions of reconstituted Proteinase K at -20°C.

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 on request.

Infectious potential of liquid waste left over after using the MagSi-cfDNA 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 Guidelines and considerations

1. Collect blood and prepare plasma samples according to the manufacturer instructions for the collection device. Use fresh plasma and serum if possible and avoid freeze-thaw cycles
2. Use fresh urine stored at 4°C and centrifuge within 24 hours of collection to avoid increase in genomic DNA and microbial growth.
3. The reagent volumes given in the protocols in Chapter 4 are per 2 mL of sample material used. Adjust volumes of Lysis Buffer CF, Proteinase K, MagSi-CF8 beads and Binding Buffer CF proportionally when processing other sample volumes.
4. Mixing and heating can also be performed by vortexing and using a water bath, but it is strongly recommended to use a thermoshaker.
5. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.
6. Do not combine components of different kits unless the lot numbers are identical.
7. Process only as many samples in parallel as the magnetic separator allows.
8. The volume of Elution Buffer can be adjusted. Although a lower volume may result in higher DNA concentrations, overall yield may be lower. The yield may also be increased by prolonging the incubation time, and with pre-heated Elution Buffer (72°C).
9. The Elution Buffer does not contain EDTA (the end user may wish to use other elution buffers containing EDTA, or Tris and EDTA, though).
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.

3.5 Magnetic separation systems

MagSi-cfDNA has been designed for manual use and automated use on the PurePrep 24 System. For manual processing in 50 mL centrifugation tubes we recommend to use the MM-Separator 50 P (REF: MDMG0015) for initial magnetic separation steps, and MM-Separator M12 + 12 P (REF: MDMG0001) for magnetic separation in 2 mL microtubes during washing and elution steps of the protocol.

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

For compatibility and availability of MagSi-cfDNA on e.g. KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™ contact magtivio. Protocols or recommendations for protocol set-up are available on request.

3.6 Product use limitations

MagSi-cfDNA 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 serum, plasma or urine 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. 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 cfDNA can be used in most genomic applications, such as sequencing, PCR, qPCR.

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 Sample pre-treatment procedures

4.1.1 Cell-Free DNA BCT® (Streck)

For preparation and storage of plasma from Cell-Free DNA BCT® tubes, please refer to the manufacturer's instructions.

4.1.2 K2-EDTA tubes

1. Centrifuge the blood collected in K2-EDTA tubes at 2,000 x g for 10 min at 4°C.
2. Transfer the plasma to a new centrifuge tube (without aspirating cells of the buffy coat).
3. Centrifuge the plasma samples at 16,000 x g for 10 min (or at 6,000 x g for 30 min) at 4°C to remove residual blood and cell debris.

4.1.3 Urine samples

1. Centrifuge the urine samples at ≥ 6,000 x g for 10 min at 4°C
2. Transfer the cell-free supernatant to a new centrifuge tube

4.2 Manual cfDNA extraction from plasma or serum samples

The extraction procedure below is scalable between 1 and 4 mL. The volumes given in the protocol below are per 2 mL sample used. Adjust volumes of Lysis Buffer CF, Proteinase K, MagSi-CF8 beads and Binding Buffer CF accordingly when processing other sample volumes.

1. Transfer **100 µL Proteinase K** to a 50 mL centrifuge tube and add **2 mL plasma or serum sample**.
2. Add **500 µL Lysis Buffer CF** 🟠 to the samples and mix well by pulse vortexing. Incubate at 56°C on a shaker for 15 min at 1000 RPM.
3. Let the sample cool down to room temperature for 5 min.

*Note: Immediately before use, resuspend **MagSi-CF8** beads by vortexing at maximum speed for 20 seconds*

4. Add **30 µL MagSi-CF8** and **4200 µL Binding Buffer CF** 🟣. Incubate on a shaker for 15 min at 1000 RPM. Make sure that the beads do not settle to the bottom of the tube during the DNA binding step.
5. Place the samples on the MM-Separator 50 P and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample tube from the MM-Separator 50 P and add **1600 µL Wash Buffer CF1** 🔵. Resuspend the magnetic beads pellet by pipetting and transfer the suspension to a 2 mL microtube. Incubate on a suitable shaker for 1 min at 1000 RPM.
7. Centrifuge for a few seconds to collect all liquid to the bottom. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Remove supernatants.
8. Remove the sample from the MM-Separator M12 + 12 P and add **1600 µL Wash Buffer CF2** 🟢. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Remove the supernatants.
9. Repeat step 8 one more time for a total of 2 washes with **1600 µL Wash Buffer CF2** 🟢.
10. Remove the sample from the MM-Separator M12 + 12 P and dry the beads on air for 10 min to evaporate the ethanol completely.
11. Add **60 µL Elution Buffer** 🟠 and incubate on a shaker for 5 min at 1000 RPM.
12. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Transfer the eluates to a new tube or microplate for analysis.

4.3 Manual cfDNA extraction from urine samples

The extraction procedure below is scalable between 1 and 10 mL. The volumes given in the protocol below are per 2 mL sample used. Adjust volumes of Lysis Buffer CF, MagSi-CF8 beads and Binding Buffer CF accordingly when processing other sample volumes.

1. Add **2 mL centrifuged urine** sample to a 50 mL centrifuge tube.

*Note: Immediately before use, resuspend **MagSi-CF8** beads by vortexing at maximum speed for 20 seconds*

2. Add **500 µL Lysis Buffer CF** 🔴, **30 µL MagSi-CF8** beads and **4200 µL Binding Buffer CF** 🟣. Incubate with shaking for 15 min at 1000 RPM. Make sure that the beads do not settle to the bottom of the tube during the DNA binding step.
3. Place the samples on the MM-Separator 50 P and wait for 2 min to collect the beads. Remove supernatants.
4. Remove the sample tube from the MM-Separator 50 P and add **1000 µL Wash Buffer CF1** 🔵. Resuspend the magnetic beads pellet by pipetting and transfer the suspension to a 1.5 mL microtube. Incubate on a suitable shaker for 1 min at 1000 RPM.
5. Centrifuge for a few seconds to collect all liquid to the bottom. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample from the MM-Separator M12 + 12 P and add **1000 µL Wash Buffer CF2** 🟢. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on the MM-Separator M12 + 12 P 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 **1000 µL Wash Buffer CF2** 🟢.
8. Remove the sample from the MM-Separator M12 + 12 P and dry the beads on air for 10 min to evaporate the ethanol completely.
9. Add **15 µL Elution Buffer** 🟠 and incubate on a shaker for 10 min at 1000 RPM.
10. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Transfer the eluates to a new tube or microplate for analysis.

4.4 Automated cfDNA extraction from plasma or serum samples on the PurePrep 24 System

4.4.1 PurePrep 24 software protocol

The PurePrep 24 protocol for MagSi-cfDNA consists of 2 parts. In Part A, the sample is incubated with Lysis Buffer CF and Proteinase K to release nucleic acids. In Part B, nucleic acids are purified from the sample by binding to magnetic beads, washing with Wash Buffer CF1 and Wash Buffer CF2, and released from the beads in Elution Buffer.

Please contact magtivio for the most recent PurePrep 24 software method files. We provide the corresponding files for direct upload on the PurePrep 24 System. A description of the method file and description of the upload procedure is included.

4.4.2 Preparation of processing plates

Plate filling for instrument set-up for **2 mL** sample volume:

Plate	Type	Reagent	Volume	Instrument Position ("Plate")
Tip Plate	PurePrep 24 DeepWell Plate	Empty, for loading Tip-Comb only	N/A	1
Sample Plate	PurePrep 24 DeepWell Plate	Part A: Proteinase K Plasma or serum Lysis Buffer CF Add after lysis incubation (Part B): MagSi-CF8 beads Binding Buffer CF	 100 µL 2000 µL 500 µL 30 µL 4200 µL	2
Wash Buffer CF1	PurePrep 24 DeepWell Plate	Wash Buffer CF1	3200 µL	3
Wash Buffer CF2 (1)	PurePrep 24 DeepWell Plate	Wash Buffer CF2	3200 µL	4
Wash Buffer CF2 (2)	PurePrep 24 DeepWell Plate	Wash Buffer CF2	3200 µL	5
Elution Buffer	PurePrep 24 DeepWell Plate	Elution Buffer	200 µL*	8

* 200 µL is the minimum specified volume to be used in the PurePrep 24 DeepWell Plate. Smaller volumes may be used but can lead to inefficient elution. A dead volume (=loss of elution buffer) due to plate geometry of up to 50 µL can be expected.

4.4.3 Detailed instructions

Follow exactly the instructions as given below. Do not change the order of reagent addition for the Sample Plate. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Add **100 µL Proteinase** into a PurePrep 24 DeepWell Plate for each sample to be processed.
2. Transfer **2 mL sample** and add **500 µL Lysis Buffer CF** 🔴.
3. Switch on the PurePrep 24 System and select Part A of the protocol. Load the Tip Plate and Sample plate onto the system (see table above for loading positions). Make sure that the plate is inserted in the correct orientation. Place the A1 well of each plate to the A1 mark on the instruments turntable.
4. Start the protocol. The samples will be incubated with mixing for 20 min at 56°C on the PurePrep 24 System.
5. Following lysis incubation, unload the sample plate and add **30 µL MagSi-CF8** beads and **4200 µL Binding Buffer CF** 🟣.
6. Prepare one plate for the 1st wash step with **Wash Buffer CF1.** Add **3200 µL Wash Buffer CF1** 🔵 to each well of the corresponding PurePrep 24 DeepWell Plate.
7. Prepare two plates for the 2nd and 3rd wash step with **Wash Buffer CF2**. Add **3200 µL Wash Buffer CF2** 🟢 to each well of the corresponding PurePrep 24 DeepWell Plates.
8. Prepare one plate for **Elution Buffer**. Add **200 µL Elution Buffer** 🟠 to each well of the corresponding PurePrep 24 DeepWell Plate.
9. Reload all plates on the PurePrep 24 System (see table above for loading positions) and select Part B of the protocol. Make sure that all plates are inserted in the correct orientation. Place the A1 well of each plate to the A1 mark on the instruments turntable
10. Start the protocol.
11. At the end of the method remove all plates from the instrument.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	Sample lysis insufficient	- Perform proteinase K digestion step. Use a suitable heater/shaker incubator. Proteinase K incubation is mandatory when using Cell-Free DNA BCT® tubes (Streck)
	Proteinase K digestion not effective	- Do not pre-mix Proteinase K with Lysis Buffer CF in order to avoid Proteinase K inactivation. This will lead to insufficient lysis and low cfDNA yield
	Insufficient amount of magnetic beads added	- Mix the bottle of magnetic beads well before adding the beads to the sample lysate - Use low retention tip or reverse pipetting technique or a pipette a small with a small excess to make sure that the required amount of beads is added to the sample lysate
	Inefficient binding to the magnetic particles	- Use correct amount of all reagents - Make sure that the magnetic beads do not settle down in the binding step. Mix with a suitable plate or tube shaker.
	Incomplete elution	- Drying of Wash Buffer CF2 may have been incomplete
	Sample contains low level of cfDNA	- Increase the sample volume
Carry over of high molecular weight DNA	Hemolytic plasma, samples to old or stored improperly	- Refer to the instructions for use of the sample tube supplier. - Do not use hemolytic samples. - Avoid carry over of buffy coat / cells when preparing plasma, use double spin protocols to prepare plasma
Problems in downstream applications/ contamination in DNA sample	Ethanol in the eluted DNA	- Remove remaining traces from Wash Buffer CF2 completely. - Increase the drying time to 15 minutes
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that supernatants from all purification steps are properly removed. - Wash buffers should be stored and used at RT
	Magnetic beads remaining in the eluate	- Place the cfDNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

magtivio B.V.

Office, Lab & Production

Daelderweg 9

6361 HK Nuth (The Netherlands)

Tel.: +31/(0)45-208 48 10

Fax: +31/(0)45-208 48 17

E-mail: info@magtivio.com

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