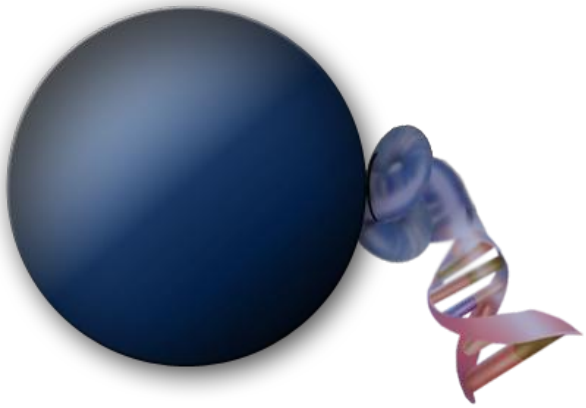
magtivio

MagSi-cfDNA

Art.No.
MDKT00220096



Product Manual

Version 1.0 | 25/11/2021

Revision history		
Revision	Release date	Remarks
1	25/11/2021	Initial release

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 or serum samples. Processing time for the preparation of 24 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 cfDNA can be used directly for downstream applications such as PCR, or any kind of enzymatic reaction.

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

MagSi-CF8 beads are optimized for use in isolating total nucleic acids. 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.

Following the lysis incubation at 56°C all other steps of the procedure are processed at room temperature.

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. 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 Basic principle

Samples are lysed under denaturing conditions by adding Lysis Buffer CF and Proteinase K at 56°C. 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	MDKT00220096
Lysis Buffer CF	50 mL
Binding Buffer CF	420 mL
Proteinase K	200 mg lyophilized (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

Reagents:

- diH_2O (to reconstitute Proteinase K)

Consumables/equipment for manual processing:

Protocol	Manual use
Containers for sample processing	50 mL Centrifugation tubes and 2 mL microtubes
Magnetic separation	MM-Separator 50 P Art. No. MDMG0015 MM-Separator M12 + 12 P Art.No. MDMG0001
Final container	1.5 or 2 mL microtubes
Lysis / mixing	Heater/shaker suitable for 50 mL tubes and 2 mL tubes (e.g. ThermoMixer C, Eppendorf)
Centrifugation	To collect liquid to the bottom after incubation with shaking

Consumables/equipment for processing on the PurePrep 24 instrument:

Product	Art. No.	Contents
PurePrep 24 Nucleic Acid Purification 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 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 for up to 1 year, but no longer than the expiry date on the label. Do not freeze!

3.2 Preparation of reagents

- Reconstitute Proteinase K:
  - Add **11 mL** of **diH_2O** 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-CF8 by vortexing for 20 seconds.

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-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 Considerations

1. Mixing and heating can also be performed by vortexing and using a water bath, but it is strongly recommended to use a heater/shaker.

2. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.

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 30-200 µL of Elution Buffer. 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).

7. The Elution Buffer does not contain EDTA (the end user may wish to use other elution buffers containing EDTA, or Tris and EDTA, though).

8. The kit is intended to be used for up to 4 mL sample volume. Deviations from the given buffer volumes, sample amounts, or different sample to buffer ratios may result in lower DNA yield due to sub-optimal binding conditions.

9. 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 (Art.No. MDMG0015) for initial magnetic bead separation steps, and MM-Separator M12 + 12 P (Art.No. MDMG0001) for magnetic bead 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 or plasma 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 materials and pre-treatment procedures

Procedure for Streck Cell-Free DNA BCT tubes:

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

If cell-free plasma is stored frozen, avoid multiple freeze-thaw cycles. Thaw plasma gently and minimize time plasma is held on ice to protect the nucleic acids from degradation. Avoid repeated freeze-thaw cycles of frozen sample material.

Procedure for K_2-EDTA tubes:

1. Centrifuge the blood collected in K2-EDTA tubes for 10 min at 2,000 x g.

2. Transfer the plasma without aspirating cells of the buffy coat layer into a fresh tube.

3. Use the obtained plasma for direct cfDNA purification or store the plasma at -20 °C or -70°C until cfDNA isolation.

Frozen samples:

If frozen samples are used: thaw frozen plasma samples prior to cfDNA isolation and centrifuge the tubes for 10 min at 4,500 x g in a table top centrifuge. Small volumes of up to 2 mL can be centrifuged for 4 min at 12,000 x g in a suitable mini centrifuge. With this centrifugation step residual cells, cell debris, and particulate matter is removed. Use the supernatant for cfDNA isolation.

4.2 Manual cfDNA extraction from human plasma or serum samples

*The extraction procedure is scalable between 1 and 4 mL. The volumes given below are **per 2 mL of sample material 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 of reconstituted Proteinase K to a 50 mL centrifuge tube and add 2 mL of sample. Finally add 500 µL of **Lysis Buffer CF**. Mix well by pulse vortexing and incubate at 56°C with shaking for 15 min at 1000 RPM.

   Processing in 50 mL disposable centrifugation tubes is possible especially for higher sample volumes of up to 5 mL.

2. After lysis incubation, let the sample cool down to room temperature. First add 30 µL **MagSi-CF8 beads** and then 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 2-4 min until the beads are attracted to the magnet. Remove supernatants.

4. Remove the sample tube from the magnetic separator and add 1600 µL **Wash Buffer CF1** to the tube. Resuspend the magnetic beads pellet. The resulting suspension can now be transferred into a 2 mL microtube. Incubate on a suitable shaker for 1 min at 1000 RPM. Centrifuge for a few seconds to collect all liquid to the bottom.

5. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads. Remove supernatants.

6. Repeat the washing step two more times with 1600 µL **Wash Buffer CF2** for each step.

7. Remove the sample tube from the magnetic separator and allow the beads in the sample tube to dry on air for 10-15 minutes in order to evaporate the ethanol traces from Wash Buffer CF2 completely.

8. Add 60 µL **Elution Buffer** to the air dried magnetic beads. Optional: Pre-heat Elution buffer to 72°C to achieve more effective DNA elution. Resuspend magnetic beads by pipetting up and down and incubate the sample tube with shaking for 10 min at 1000 RPM.

9. Place the samples on the MM-Separator M12 + 12 P and wait for 1 min to collect the beads.

10. Transfer the eluates to a new tube. The DNA in the eluate is now ready to use. For cfDNA quantification use fluorescent dye based methods (e.g. Qubit Highsense assay or qPCR based methods)

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

4.3.1 PurePrep 24 software protocol

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.

4.3.2 Preparation of processing plates

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

Plate	Type	Reagent	Volume
Sample Plate	PurePrep 24 DeepWell Plate	Sample Lysis Buffer CF Proteinase K solution *Add after lysis incubation:* MagSi-CF8 beads Binding Buffer CF	2000 µL 500 µL 100 µL 30 µL 4200 µL
Wash Buffer CF1	PurePrep 24 DeepWell Plate	Wash Buffer CF1	3200 µL
Wash Buffer CF2 - 1st	PurePrep 24 DeepWell Plate	Wash Buffer CF2	3200 µL
Wash Buffer CF2 - 2nd	PurePrep 24 DeepWell Plate	Wash Buffer CF2	3200 µL
Elution Buffer	PurePrep 24 DeepWell Plate	Elution Buffer	200 µL*)
Tip plate	PurePrep 24 DeepWell Plate	Empty, for loading Tip-Comb only	N/A

*) 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.

4.3.3 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. Transfer **2 mL** of each sample into a PurePrep 24 DeepWell Plate.
2. Add **500 µL Lysis Buffer CF** and **100 µL Proteinase K**. Load the plate on the PurePrep 24 instrument and start the protocol. The samples will be incubated with mixing for 20 min at 56°C on the PurePrep 24 instrument.
3. Following lysis incubation, unload the sample plate and add to the lysed samples: 1st: **30 µL MagSi-CF8** magnetic beads and then 2nd: **4200 µL Binding Buffer CF**.
4. 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.
5. 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.
6. Prepare one plate for **Elution Buffer**. Add **200 µL Elution Buffer** to each well of the corresponding PurePrep 24 DeepWell Plate.
7. Reload all plates on the PurePrep 24 System (see table above for loading positions) and select the protocol. Make sure that all plates are inserted 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. Start the protocol.
9. 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 Streck Cell-Free DNA BCT tubes (or similar)
	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. Alternatively mix by repeated pipetting up and down for
	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.

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