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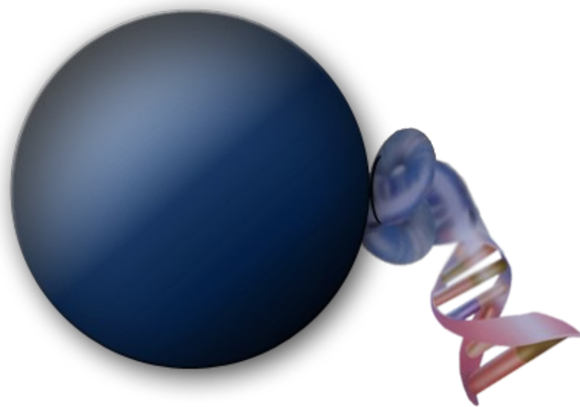
MagSi-NA Pathogens

Art.No.

MDKT00210096

MDKT00210960



Product Manual

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1. General Information

1.1 Intended Use

MagSi-NA Pathogens 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 nucleic acids (DNA and RNA) from a wide range of samples. Processing time for the preparation of 96 samples is about 40 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 nucleic acids can be used directly as template for downstream applications such as PCR, qPCR, qRT-PCR or any kind of enzymatic reaction.

MagSi-NA Pathogens is suitable for use with blood samples, liquid samples (e.g. plasma, serum, urine, swab washes), tissue samples, feces. For details on the individual procedures for sample pre-treatment see below.

MagSi-NA Pathogens magnetic 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.

1.2 Kit specifications

The kit provides reagents for extraction of total nucleic acids from 200 µL liquid sample or 200 µL homogenized tissue samples, cells or suspended feces. Total nucleic acids are finally eluted in a volume of 100 µL Elution Buffer.

The obtained nucleic acids should be used for qPCR, qRT-PCR immediately after extraction. Storage at <-20°C is recommended for later analysis.

1.3 Basic principle

Samples are lysed under denaturing conditions by adding Lysis Buffer PA1 and Proteinase K. During the incubation the released nucleic acids can bind to the MagSi-PA VII magnetic beads. After magnetic separation and discard of the supernatant, the beads are washed three times using alcoholic buffers (Wash Buffers I and II) to remove contaminants and salts. A drying step makes sure all traces of ethanol from the final wash steps 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

	96 preps MDKT00210096	960 preps MDKT00210960
Lysis Buffer PA1	20 mL	200 mL
Binding Buffer U1	40 mL	400 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
Proteinase K	20 mg (for 1 mL working solution)	200 mg (for 10 mL working solution)
Poly-A-RNA	0.3 mg	10 x 0.3 mg
Poly-A-RNA Buffer	0.5 mL	10 x 0.5 mL
MagSi-PA VII	2 mL	20 mL

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

Reagents:

- molecular biology grade (nuclease free) water to reconstitute Proteinase K
-

Consumables/equipment:

Protocol	Manual use	Automated use
Sample containers	1.5 or 2 mL microtubes	Recommended: Riplate®SW 96, PP, 2 mL, (Ritter, 43001–0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0 mL, (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
Mixing	Tube Vortexer	Microplate shaker (min. 1000 RPM)

Consumables for processing on the KingFisher™ Flex instrument

Product	Art. No.	Contents
2 mL Deepwell Plate with square wells for KingFisher™	MDPL00200060	60 pieces
200 µL square-well Elution Plate for KingFisher™	MDPL00190060	60 pieces
96 well Tip-Comb for KingFisher™	MDPL00210060	60 pieces

3. Kit usage

3.1 Storage Conditions

All kit components including **Proteinase K** (lyophilized), **Poly-A-RNA** (lyophilized) and **MagSi-PA VII** can be stored at room temperature. Store ready solutions of Proteinase K and Poly-A-RNA in suitable aliquots 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 Proteinase K:

  MDKT00210096 (96 preps), add 1 mL of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. Store solution of Proteinase K in aliquots at -20°C.

  MDKT00210960 (960 preps), add 10 mL of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. Store solution of Proteinase K in aliquots at -20°C.

- Reconstitute Poly-A-RNA:

  Add 120 µL of **Poly-A-RNA Buffer** to each vial of **Poly-A-RNA** and vortex to dissolve. Store solutions of Poly-A-RNA in aliquots 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-PA VII beads by vortexing for 20 seconds.

- Samples should be thoroughly mixed before use.

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 from AMSBIO.

Infectious potential of liquid waste left over after using the MagSi-NA Pathogens 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 nucleic acids concentrations, overall yield may be lower. The yield may also be increased by prolonging the incubation time, and with pre-heated Elution Buffer (56°C).
6. The Elution Buffer does not contain EDTA.
7. Avoid samples containing coagulates or precipitates, as this may result in poor results or quality. Centrifuge samples before use.
8. The kit is compatible with whole blood treated with EDTA and citrate. Heparin is co-isolated and may interfere with subsequent PCR analyses.
9. 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.

3.5 Magnetic Separation systems

MagSi-NA Pathogens 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.N o. MDMG0013).

For use with other magnetic separators, please contact the technical support via AMSBIO.com

MagSi-NA Pathogens is compatible with KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Protocols and consumables 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 Product use limitations

MagSi-NA Pathogens 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 specified. It is recommended to check the suitability of the purified nucleic acids for each selected qPCR / qRT-PCR assay.

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. 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 nucleic acids can be used in most genomic applications, such as 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

Preparation of the **Lysis Master Mix**

For each sample mix 200 µL Lysis Buffer PA1 with 1 µL reconstituted Poly-A-RNA and 10 µL of reconstituted Proteinase K solution (20 mg/mL). Prepare an excess of the Lysis Master Mix to compensate for pipetting inaccuracy especially when using multichannel pipettes etc. Use the Lysis Master Mix immediately after preparation.

Recommendations for sample pre-treatment

Sample material	Pre-treatment procedure	Further processing
Liquid samples (e.g. plasma, serum, blood, swab wash solutions)	n/a	Use up to 200 µL of the liquid sample and add 211 µL Lysis Master Mix
Feces / stool	Add 1.5 mL molecular biology grade water to a pea-size amount of feces. Mix well by vortexing. Spin down at low g-forces to remove remaining particulate sample residuals. Use 200 µL of the suspension for further processing.	Use up to 200 µL of the centrifuged suspension and add 211 µL Lysis Master Mix
Tissue samples	Mechanically homogenize <30 mg of tissue sample in 500 µL molecular biology grade water using suitable devices (bead beater). Spin down for 1 min at 8,000 x g to remove debris. Use 200 µL of the suspension for further processing.	Use up to 200 µL of the centrifuged suspension and add 211 µL Lysis Master Mix
Dry swabs	Add 500 µL molecular biology grade water to dried swab and shake/vortex vigorously for some minutes. Take 200 µL for further processing	Use 200 µL of the wash solution and add 211 µL Lysis Master Mix

For samples not mentioned in the table above please contact AMSBIO for support protocols.

4.2 Manual processing of 200 µL liquid or pre-treated samples

Before starting:

- *Check if Lysis Master Mix was prepared according to section 4.1.*
- *Pre-treat of samples (if required) according to section 4.1*
- *Vortex magnetic beads thoroughly into a homogeneous suspension.*

This protocol is intended for manual use of the kit. It can also be used as a guideline to set up an automated procedure on liquid handling instruments. For this suitable 96-well plates and accessories can be used. Make sure that the liquid handling devices is equipped with the required devices (shaker, incubator, magnetic separator etc.).

1. Transfer **200 µL sample** into a microtube. If the volume is lower than 200 µL, bring the volume up to 200 µL with 1 x PBS buffer or molecular biology grade water.

2. Add **211 µL Lysis Master Mix**. Mix immediately and incubate on a shaker for **10 min with shaking at 1000 RPM**. Spin down briefly to collect any sample from the microtube lid.

3. Add **20 µL MagSi-PA VII** beads and **400 µL Binding Buffer U1**. Mix and incubate on a shaker for **5 min with shaking at 1000 RPM**. Spin down briefly to collect any sample from the microtube lid.

4. Place the samples on the magnetic separator and wait at least 1 minute to collect the beads. Remove supernatants without disturbing the attracted magnetic bead pellet.

5. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer I** to the tubes. Resuspend the beads by incubation of the samples on a shaker for **1 min at 1000 RPM** (alternatively pipette the wash buffer up and down until the beads are resuspended completely). Place the samples in a magnetic separator and wait at least 1 minute to collect the beads. Remove the supernatants without disturbing the attracted bead pellet.

6. Repeat step 5 one more time with **800 µL Wash Buffer I** and one more time with **800 µL Wash Buffer II.**

7. Dry the beads on air for **10 min** to evaporate the ethanol completely. If necessary briefly spin down and remove any buffer residues before drying the attracted magnetic beads.

8. Remove the samples from the magnetic separator and add **100 µL Elution Buffer.** Resuspend the attracted magnetic beads by repeated pipetting up and down. **Incubate** on a shaker for **10 min at 1000 RPM.**

9. Place the tubes in a magnetic separator and wait at least 1 minute to collect the beads. Transfer the eluates to new tubes. The purified nucleic acids in the eluate are 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 nucleic acids can be eluted with a lower volume of Elution Buffer (depending on the expected yield). The minimum volume for elution is 30 µL and this can reduce the yield. If a large amount of nucleic acids is expected, the volume of Elution Buffer can be increased.*

4.3 Protocol for the KingFisher Flex™ Magnetic Particle Processor

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. 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.

4.3.1 Preparation of processing plates

Initial plate filling for instrument set-up:

Plate	Type*)	Reagent	Volume
Sample Plate	2 mL Deepwell Plate with square wells for KingFisher™	(pre-treated) sample adjusted to 200 µL Lysis Master Mix MagSi PA VII beads Binding Buffer U1	200 µL 211 µL 20 µL 400 µL
Wash Buffer I 1st	2 mL Deepwell Plate with square wells for KingFisher™	Wash Buffer I	800 µL
Wash Buffer I 2nd	2 mL Deepwell Plate with square wells for KingFisher™	Wash Buffer I	800 µL
Wash Buffer II	2 mL Deepwell Plate with square wells for KingFisher™	Wash Buffer II	800 µL
Elution Buffer	200 µL square-well Elution Plate for KingFisher™	Elution Buffer	100 µL
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™	Empty, for loading Tip-Comb only	N/A

To be added after the initial lysis step on the KingFisher™ Flex magnetic particle processor

*) Suitable plates can be purchased at AMSBIO (see section 2.2). We strongly recommend to use only the plates which are intended to use on the KingFisher™ magnetic particle processor. Using unsuitable plates may result in extraction failure or instrument damage.

4.3.2 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. Prepare samples according to section 4.1. Add **200 µL of the sample** to each well of the Sample Plate. If the volume is lower than 200 µL, bring the volume up to 200 µL with 1 x PBS buffer or molecular biology grade water.

2. Add **211 µL of Lysis Master Mix** to each well of the Sample Plate. Mix by pipetting up and down until a homogeneous mixture is visible (typically 3 – 4 times). Incubate on a suitable plate shaker for 10 min with shaking at 1000 rpm. Continue with preparing the remaining plates before continuing with the sample plate.

3. Prepare two plates for the 1st and 2nd wash steps with **Wash Buffer I**. Add **800 µL Wash Buffer I** to each well of the corresponding deep-well plates.

4. Prepare one plates for the 3rd wash step with **Wash Buffer II.** Add **800 µL Wash Buffer II** to each well of the corresponding deep-well plate.

5. Prepare one plate for **Elution Buffer** Add **100 µL Elution Buffer** to each well of the corresponding plate.

6. Add **20 µL of MagSi-PA VII beads** to each well of the Sample Plate.

7. Add **400 µL of Binding Buffer U1** to each well of the Sample Plate.

8. Switch on the KingFisher™ Flex magnetic particle processor and select the **"MagSi-NA Pathogens"** protocol from the user defined protocols

9. Start the protocol.

10. 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 all plates are inserted in the same orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable.

11. At the end of the method remove all plates from the instrument. Follow the information on the instrument display.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low nucleic acid yield	Insufficient sample lysis	- Optimize sample pre-treatment, make sure that Lysis Master Mix includes Proteinase K
	Inefficient binding to the magnetic particles	- Use correct amount of all reagents - Increase mixing steps incubation time for binding step - Mix sample during lysis / binding incubation
	Insufficient washing procedure	- Make sure that beads are completely resuspended in the washing steps.
	Incomplete elution	- Drying of Wash Buffer II may have been incomplete - Completely resuspend the beads in the elution step.
Problems in downstream applications / contamination in DNA sample	Ethanol in the eluted DNA	- Increase the drying time to 15 minutes
	Salt in the eluate	- Make sure that all supernatants are properly removed. - Avoid carry-over of Lysis Master Mix , Binding Buffer or Wash Buffers to the eluate.
	Magnetic beads remaining in the eluate	- Place the tubes with eluates in the magnetic separator again, and transfer the supernatant to a new container. High amounts of co-purified genomic DNA (e.g. for cell and tissue samples) may cause high viscosity of the eluate and force incomplete bead separation in the elution step. Use higher volume of elution buffer and/or reduce sample input.