magtivio

rQ MagSi-NA Pathogens

Art.No.

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MDKT00211096PF
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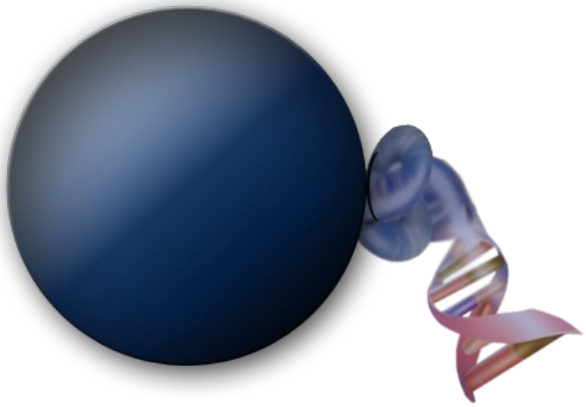

Product Manual

Version 1.0 | 17/11/2021

Revision history		
Revision	Date of release	Remarks
1	17/11/2021	Initial release

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Table of Contents

1. General Information

1.1 Intended Use

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

The kit is intended for automated isolation of nucleic acids (DNA and RNA) from a wide range of samples using magnetic particle processors, such as the PurePrep 96 Nucleic Acid Purification System or KingFisher™ Flex 96. Processing time for the preparation of 96 samples is about 20 minutes. The kit is provided in prefilled format and 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.

rQ 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.

rQ MagSi-NA Pathogens magnetic beads are optimized for use in isolating total nucleic acids and minimum collection time with high separation efficiency. The beads are supplied in diluted in a storage buffer in prefilled format and are transferred to the sample by the magnetic particle processing instrument.

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, Proteinase K, Poly-A-RNA and Binding Buffer U1 are added to the rQ Lysis Buffer PA1 plate. All prefilled plates containing samples and extraction buffers, and the 96 Tip-Comb, are placed on the instrument and the automated protocol is started. During a short incubation, samples are lysed and nucleic acids bind to the magnetic beads. Afterwards, the magnetic beads are collected to the tip comb and transferred into three subsequent wash plates containing alcoholic buffers (Wash Buffers I and II) before air-drying and finally eluting nucleic acids into Elution Buffer. Purified nucleic acids are eluted with low-salt Elution Buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	1x96 preps MDKT00210196PF	10x96 preps MDKT00211096PF	50x96 preps MDKT00215096PF
rQ Lysis Buffer PA1	1 Unit(s)	10 Unit(s)	50 Unit(s)
rQ Wash Buffer I	2 Unit(s)	20 Unit(s)	100 Unit(s)
rQ Wash Buffer II	1 Unit(s)	10 Unit(s)	50 Unit(s)
rQ Elution Buffer	1 Unit(s)	10 Unit(s)	50 Unit(s)
rQ MagSi-PA VII	1 Unit(s)	10 Unit(s)	50 Unit(s)
96 well Tip-Comb for KingFisher™/PurePrep 96	2 Unit(s)	10 Unit(s)	50 Unit(s)
Proteinase K	20 mg (for 1.1 mL working solution)	200 mg (for 11 mL working solution)	1000 mg (for 55 mL working solution)
Binding Buffer U1	40 mL	400 mL	2 x 1000 mL
Poly-A-RNA	0.3 mg (for 120 µL working solution)	3 mg (for 1.2 mL working solution)	15 mg (for 6 mL working solution)
Poly-A-RNA Buffer	0.5 mL	5 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:

- Vortexer
- Centrifuge for 96-deepwell microtiter plates
- Pipettes and pipette tips for addition of samples, Binding Buffer U1, reconstituted Proteinase K and Poly-A-RNA
- PurePrep 96 Nucleic Acid Purification System, KingFisher Flex 96 or comparable instruments
- Depending on sample type used, a thermoshaker (e.g. ThermoMixer C) for sample pretreatment / lysis incubation

3. Kit usage

3.1 Storage Conditions

All kit components including **Proteinase K** (lyophilized) and **Poly-A-RNA** (lyophilized) can be stored at room temperature. When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Preparation of reagents

3.2.1 Preparation of prefilled plates

- If there is any precipitate present in the buffers, warm the prefilled plates to 25-37°C to dissolve the precipitate before use.
- Before pealing off the seal from the prefilled plates, spin down briefly at low centrifugal force (1000 x g) for 30-60 seconds.

3.2.2 Reconstitute Proteinase K

- MDKT00210196PF (1x96 preps), add **1.1 mL** of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. Store solutions of Proteinase K in aliquots at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- MDKT00211096PF (10x96 preps), add **11 mL** of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. For aliquotation per 96 samples, make aliquots of 1 mL and store solutions at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- MDKT00215096PF (50x96 preps), add **55 mL** of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. For aliquotation per 96 samples, make aliquots of 1 mL and store solutions at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.

3.2.3 Reconstitute Poly-A-RNA

- MDKT00210196PF (1x96 preps), add **120 µL** of **Poly-A-RNA Buffer** to the vial of **Poly-A-RNA (0.3 mg)** and vortex to dissolve. Store solutions of Poly-A-RNA at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- MDKT00211096PF (10x96 preps), add **1.2 mL** of **Poly-A-RNA Buffer** to the vial of **Poly-A-RNA (3 mg)** and vortex to dissolve. For aliquotation per 96 samples, make aliquots of 110 µL and store solutions at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- MDKT00215096PF (50x96 preps), add **6 mL** of **Poly-A-RNA Buffer** to the vial of **Poly-A-RNA (15 mg)** and vortex to dissolve. For aliquotation per 96 samples, make aliquots of 110 µL and store solutions at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.

3.2.4 Preparation of Binding Premix

For Protocol with off-line lysis incubation (see section 4.4):

- Prepare the Binding Premix immediately before use
- Per 96 samples, mix 40 mL Binding Buffer U1 and 100 µL Poly-A-RNA
- Mix by vortexing

For Protocol with single step lysis-binding incubation (see section 4.5):

- Prepare the Binding Premix immediately before use
- Per 96 samples, mix 40 mL Binding Buffer U1, 1000 µL Proteinase K and 100 µL Poly-A-RNA
- Mix by vortexing

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.

Infectious potential of liquid waste left over after using the rQ 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 degradation of nucleic acids, change pipette tips after each use and use nuclease-free filter-tips.
2. Avoid leaving prefilled plates, bottles or tubes open to prevent contamination or evaporation of the kit reagents.
3. The elution can be done in smaller volumes of Elution Buffer. The 96 DWP with Elution Buffer contains 100 µL per well. If desired, a minimum volume of 70 µL can be used by removing 30 µL from each well. Due to the heating in the elution step, a volume of approximately 50 µL remains, which is the minimum to allow effective bead collection from the elution plate. Although removing Elution Buffer may result in higher nucleic acids concentrations, overall yield may be lower.
4. The Elution Buffer contains 10 mM Tris pH 8. It does not contain EDTA.
5. Avoid samples containing coagulates or precipitates, as this may result in poor results or quality. Centrifuge samples before use.
6. The kit is compatible with whole blood treated with EDTA and citrate. Heparin is co-isolated and may interfere with subsequent PCR analyses.

3.5 Magnetic Separation systems

rQ MagSi-NA Pathogens has been designed for use on PurePrep 96 Nucleic Acid Purification System and KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™, or comparible devices. Protocols are available on request.

3.6 Instrument protocol files

Please contact magtivio for the most recent PurePrep 96 protocol files or BindIt software method files. We provide the corresponding files for direct import to the PurePrep 96 Nucleic Acid Purification System or uploading to the KingFisher™ magnetic particle processors through the BindIt software. Refer to the PurePrep 96 user instructions or BindIt software manual regarding the import/upload procedure of the supplied files to the instrument.

3.7 Sample materials, pretreatment and lysis procedure

Recommendations for sample pre-treatment and lysis

Sample material	Pre-treatment procedure and lysis procedure
Saliva, swab wash solutions	n/a Note: Lysis/binding can also be performed in a single step on the magnetic particle processor
Plasma, serum, blood	n/a Off-line lysis step with Proteinase K needed before addition of Binding Premix and automated nucleic acid purification
Dry swabs without transport media	Add an appropriate volume of molecular biology grade water to dried swab (swab should be completely submerged) and shake/vortex vigorously for 15 minutes. Take 200 µL for further processing Note: Lysis/binding can also be performed in a single step on the magnetic particle processor
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. Off-line lysis step with Proteinase K needed before addition of Binding Premix and automated nucleic acid purification
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. Off-line lysis incubation with Proteinase K needed before addition of Binding Premix and automated nucleic acid purification

For samples not mentioned in the table above please contact magtivio for support protocols. Samples should be thoroughly mixed before use.

4. Procedure for the PurePrep 96 System

4.1 Protocol selection

Depending on the type of sample, a selection must be made for the appropriate protocol, using an off-line lysis incubation or a single-step lysis/binding procedure (see section 3.7)

4.2 Importing the instrument protocol (if needed)

To save the protocol to your PurePrep 96 Nucleic Acid Purification System:

1. Plug in the USB drive
2. Switch on the instrument
3. From the main menu select “Settings”
4. Select “Im.&export”, and “Import”
5. Select the file to be imported from the list or select all files
6. Select “Import”, file(s) will be uploaded to the instrument now
7. Select “Back” two times to return to the main menu
8. Select “Manage Prog.”
9. Select the protocol to create a shortcut

4.3 Before starting

- Switch on the PurePrep 96 System and turn on ultraviolet disinfection for 20 min before use.
- Pre-treat samples (if required) according to section 3.7.
- Make sure to follow the instructions for preparation of reagents according to section 3.2.

4.4 Protocol with off-line lysis incubation

1. Carefully peel off the seal from all plates.
2. Add samples to the rQ Lysis Buffer PA1 plate and add 10 µL Proteinase K to each sample
3. Place sample plate on a plate shaker for 10 min at room temperature
4. Remove the sample plate from the shaker and add 400 µL Binding Premix (see section 3.2.4).
5. Put all plates on the corresponding positions in the instrument (see table below) and place a Tip-Comb in the DWP with MagSi-PA VII. Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions. 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

Plate (position) in the protocol	Component(s)	Content(s)
2	96 DWP with Lysis Buffer PA1 Sample Proteinase K *After lysis incubation:* Binding Premix	200 µL 200 µL (added by user) 10 µL (added by user) 400 µL (added by user)
3	96 DWP with MagSi-PA VII / Tip-Comb	500 µL
4	96 DWP with Wash Buffer I	800 µL
5	96 DWP with Wash Buffer I	800 µL
6	96 DWP with Wash Buffer II	800 µL
8	96 DWP with Elution Buffer	100 µL

6. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
7. At the end of the run remove all plates from the instrument

4.5 Protocol with single step lysis-binding incubation

1. Carefully peel off the seal from all plates.

2. Add samples to the rQ Lysis Buffer PA1 plate and add 411 µL Binding Premix to each sample (see section 3.2.4)

3. Put all plates on the corresponding positions in the instrument (see table below) and place a Tip-Comb in the DWP with MagSi-PA VII. Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions. 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.

Plate (position) in the protocol	Component(s)	Content(s)
2	96 DWP with Lysis Buffer PA1 Sample Binding Premix	200 µL 200 µL (added by user) 411 µL (added by user)
3	96 DWP with MagSi-PA VII / Tip-Comb	500 µL
4	96 DWP with Wash Buffer I	800 µL
5	96 DWP with Wash Buffer I	800 µL
6	96 DWP with Wash Buffer II	800 µL
8	96 DWP with Elution Buffer	100 µL

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.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low nucleic acid yield	Ineffective sample lysis	- Optimize sample pre-treatment, make sure that Proteinase K is added to sample
	Incomplete elution	- Do not reduce elution volume by removal of Elution Buffer and/or add nuclease-free water to increase elution volume up to 200 µL
Problems in downstream applications / contamination in DNA sample	Magnetic beads remaining in the eluate. 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.	- Place the plates with eluates in a 96-well magnetic separator, and transfer the supernatant to a new container. - Do not reduce the volume of elution buffer by removing part of it. Reduce sample input and/or increase elution volume by adding nuclease-free water

6. Product use limitations

rQ 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. 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 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.

7. Bulk packages

All components included in the prefilled kits are available on request in bulk supply.

magtivio B.V.

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