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rQ MagSi-NA Pathogens

Art.No.

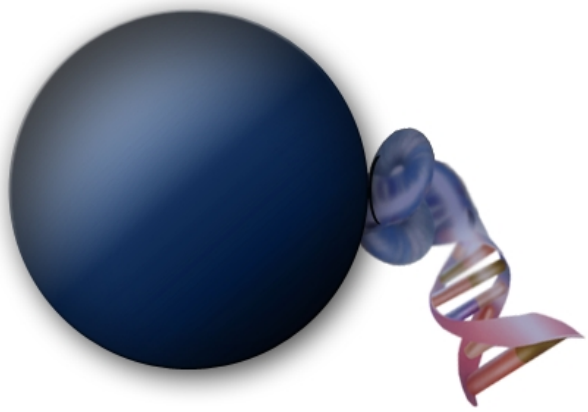
MDKT00210216PF (2 x 16 preps)

MDKT00210616PF (6 x 16 preps)

MDKT00210096PF (1 x 96 preps)

MDKT00211096PF (10 x 96 preps)

MDKT00215096PF (50 x 96 preps)



Product Manual

Version 3.0 | 21/09/2022

Revision history		
Revision	Date of release	Remarks
1	17/11/2021	Initial release
2	10/05/2022	Addition of MDKT00210216 and MDKT00210616, text corrections
3	21/09/2022	Text corrections

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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 viral nucleic acids from a wide range of samples using magnetic particle processors, such as the PurePrep 96, PurePrep 32 and PurePrep 16 Nucleic Acid Purification Systems, or KingFisher™ Flex 96. Processing time for the preparation of 96 samples is about 20 minutes, depending on the instrument used. 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 swab samples, saliva, whole blood and plasma.

rQ MagSi-VII magnetic beads are optimized for use in isolating total nucleic acids and minimum collection time with high separation efficiency. The beads are supplied 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. 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 and Poly-A-RNA are added to the rQ Lysis Buffer PA1 plate. After an optional offline pre-lysis step, Binding Buffer U1 is added to establish conditions for binding nucleic acids to magnetic beads. Prefilled plates containing samples and extraction buffers, and the 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

Kits for PurePrep 96 Nucleic Acid Purification System or KingFisher™ Flex 96:

	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

Kits for PurePrep 32 and PurePrep 16 Nucleic Acid Purification Systems:

	2x16 preps MDKT00210216PF	6x16 preps MDKT00210616PF
rQ Pathogens extractions plate	2 Unit(s)	6 Unit(s)
8 rod Tip comb	2 Unit(s)	6 Unit(s)
Proteinase K	20 mg (for 1.1 mL working solution)	20 mg (for 1.1 mL working solution)
Binding Buffer U1	40 mL	40 mL
Poly-A-RNA	0.3 mg (for 120 µL working solution)	0.3 mg (for 120 µL working solution)
Poly-A-RNA Buffer	0.5 mL	0.5 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, PurePrep 32 or PurePrep 16 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.

It is recommended not to directly stack the plates on top of each other to avoid damaging the seal, which could lead to evaporation of the buffers and thus to a lower efficiency of the kit.

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 seconds.

3.2.2 Reconstitute Proteinase K

- MDKT00210216PF (2x16 preps), MDKT00210616PF (6x16 preps), 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

- MDKT00210216PF (2x16 preps), MDKT00210616PF (6x16 preps), 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 sections 3.7, 4.3 and 4.5):

- Prepare the Binding Premix immediately before use:
  - Per 96 samples, mix 40 mL Binding Buffer U1 and 100 µL Poly-A-RNA
  - Per 16 samples, mix 6.8 mL Binding Buffer U1 and 17 µL Poly-A-RNA
  - Mix by vortexing

For Protocol with single step lysis-binding incubation (see sections 3.7, 4.4 and 4.6):

- 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
  - Per 16 samples, mix 6.8 mL Binding Buffer U1, 170 µL Proteinase K and 17 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 kit contains 100 µL Elution Buffer 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, PurePrep 32 and PurePrep 16 Nucleic Acid Purification Systems as well as KingFisher™ Flex 96 Magnetic Particle Processor by Thermo Scientific™, or comparable devices. Protocols are available on request.

3.6 Instrument protocol files

Please contact magtivio for the most recent PurePrep protocol files or BindIt software method files. We provide the corresponding files for direct import to the PurePrep Nucleic Acid Purification Systems or uploading to the KingFisher™ magnetic particle processors through the BindIt software. Refer to the PurePrep 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	Pretreatment: Not required Lysis: Lysis/binding can also be performed in a single step on the magnetic particle processor
Plasma, serum, whole blood	Pretreatment: Not required Lysis: 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

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

4. Extraction protocols

4.1 Protocol selection

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

The PurePrep 32 System allows for use of 1 or 2 plates, for a total of 16 or 32 extractions. Using fewer than 16 samples per plate is not recommended, as the plates cannot be reused.

The PurePrep 96 System allows for use of 1 sample plate, for a total of 96 extractions. Using fewer than 96 samples per plate is not recommended, as the plates cannot be reused.

4.2 Before starting

- Switch on the PurePrep 32 or PurePrep 16, or 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.

4.3 PurePrep 16 and PurePrep 32 protocol (off-line lysis)

Plate overview:

Plate column ("Well") in the protocol	Component(s)	Content(s)
1 / 7	rQ 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)
2 / 8	rQ MagSi-PA VII	500 µL
3 / 9	rQ Wash Buffer I	800 µL
4 / 10	rQ Wash Buffer I	800 µL
5 / 11	rQ Wash Buffer II	800 µL
6 / 12	rQ Elution Buffer	100 µL

1. Spin down the rQ Pathogens Extraction plate briefly at low centrifugal force (<1000 x g) for ~30 seconds.
2. Carefully peel off the seal from the rQ Pathogens Extraction plate(s).
3. Add samples to column 1 and 7 in the rQ Pathogens Extraction plate(s) and add 10 µL Proteinase K to each sample
4. Place sample plate(s) on a plate shaker for 10 min at room temperature
5. Remove the plate(s) from the shaker and add 400 µL Binding Premix (see section 3.2.4) to plate column 1 and 7.
6. Put the plate(s) on the corresponding positions in the instrument (see table below) and place the 8 rod Tip combs in the holder. Make sure that the plate(s) are loaded in the correct orientation (column 1 on the left).

<u>Important note: Even when using only 8 or less samples, load two 8 rod Tip combs per plate! Magnet rods without Tip comb will get contaminated with beads/buffers.</u>

7. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
8. At the end of the run remove the tip comb(s) and plate(s) from the instrument.

4.4 PurePrep 16 and PurePrep 32 protocol (single step lysis-binding incubation)

Plate overview:

Plate column ("Well") in the protocol	Component(s)	Content(s)
1 / 7	rQ Lysis Buffer PA1 Sample Binding Premix	200 µL 200 µL (added by user) 411 µL (added by user)
2 / 8	rQ MagSi-PA VII	500 µL
3 / 9	rQ Wash Buffer I	800 µL
4 / 10	rQ Wash Buffer I	800 µL
5 / 11	rQ Wash Buffer II	800 µL
6 / 12	rQ Elution Buffer	100 µL

1. Spin down the rQ Pathogens Extraction plate briefly at low centrifugal force (<1000 x g) for ~30 seconds.

2. Carefully peel off the seal from the rQ Pathogens Extraction plate(s).

3. Add samples and add 411 µL Binding Premix (see section 3.2.4) to columns 1 and 7 in the rQ Pathogens Extraction plate(s).

4. Put the plate(s) on the corresponding positions in the instrument (see table below) and place the 8 rod Tip combs in the holder. Make sure that the plate(s) are loaded in the correct orientation (column 1 on the left).

Important note: Even when using only 8 or less samples, load two 8 rod Tip combs per plate! Magnet rods without Tip comb will get contaminated with beads/buffers.

5. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol

6. At the end of the run remove the tip comb(s) and plate(s) from the instrument.

4.5 PurePrep 96 protocol (off-line lysis)

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 rQ 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	rQ 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	rQ MagSi-PA VII / Tip-Comb	500 µL
4	rQ Wash Buffer I	800 µL
5	rQ Wash Buffer I	800 µL
6	rQ Wash Buffer II	800 µL
8	rQ 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

4.6 PurePrep 96 protocol (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 rQ 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	rQ MagSi-PA VII / Tip-Comb	500 µL
4	rQ Wash Buffer I	800 µL
5	rQ Wash Buffer I	800 µL
6	rQ Wash Buffer II	800 µL
8	rQ 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 may cause high viscosity of the eluate and incomplete bead separation in the elution step.	- Place the plates with eluates in a 96-well magnetic separator, and transfer the sample supernatant to a new container. - Do not reduce the volume of rQ 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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