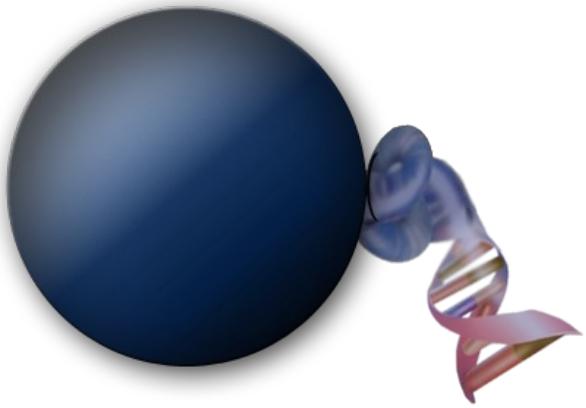
magtivio

MagSi-NA Pathogens MSP

Art.No.

MDKT0021P06K



Product Manual

Version 1.0 | 30/06/2021

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1. General Information

1.1 Intended Use

MagSi-NA Pathogens MSP 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 respiratory samples. The sample preparation procedure includes magnetic sample pooling of up to 6 samples without diluting the isolated nucleic acids. The obtained nucleic acids can be used directly as template for downstream applications such as PCR, qPCR, RT-qPCR or any kind of enzymatic reaction.

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. Processing time for the preparation of 6 x 96 samples is about 50 minutes after preparing the sample plates and aliquoting the kit components.

MagSi-NA Pathogens MSP magnetic beads are optimized for use in isolating total nucleic acids from up to 6 samples. 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 up to 6000 respiratory swabs or saliva samples of 200 µL. 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

Up to 6 x 96 samples are added to 6 deepwell microtiter plates. Samples are lysed under denaturing conditions by adding Lysis Working Solution, including Lysis Buffer PA1, Proteinase K and Poly-A-RNA. Magnetic beads MagSi-MSP and Binding Buffer U1 are added to each sample plate and will bind nucleic acids in a serial manner, by incubation and transfer to a new sample plate, until all sample plates have been incubated and magnetic beads are collected from each plate. Afterwards, the magnetic beads are washed in 3 deepwell microplates containing alcoholic buffers (Wash Buffers I and II). Finally nucleic acids collected from up to 6 samples are released into an elution plate with low-salt Elution Buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	Up to 6000 samples MDKT0021P06K
Lysis Buffer PA1	2 x 600 mL
Binding Buffer U1	3 x 800 mL
Wash Buffer I	2 x 800 mL
Wash Buffer II	800 mL
Elution Buffer	200 mL
Proteinase K	1250 mg (for 62.5 mL working solution)
Poly-A-RNA	18 mg (for 7.2 mL working solution)
Poly-A-RNA Buffer	20 mL
MagSi-MSP	120 mL

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

Equipment & consumables:

Name	Cat.No.
Vortexer for resuspending beads and mixing working solutions	-
PurePrep 96 magnetic particle processor	AS00001
2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	MDPL00200050
200 µL square-well Elution Plate for KingFisher™/PurePrep 96	MDPL00190060
96 well Tip-Comb for KingFisher™/PurePrep 96	MDPL00210060

Reagents:

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

3. Kit usage

3.1 Storage Conditions

All kit components including **Proteinase K** (lyophilized), **Poly-A-RNA** (lyophilized) and **MagSi-MSP** 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

- If there is any precipitate present in the buffers, warm the buffer to 25-37°C to dissolve the precipitate before use.
- Reconstitute **Proteinase K**:
  - Add 62.5 mL of **molecular biology grade water** to the vial of **Proteinase K** and vortex to dissolve. For aliquotation per 96 samples, make aliquotes of 1.05 mL and store solutions at -20°C. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- Reconstitute **Poly-A-RNA**:

  Add 7.2 mL of **Poly-A-RNA Buffer** to the vial of **Poly-A-RNA (18 mg)** and vortex to dissolve. Store solutions of Poly-A-RNA at -20°C. Avoid repeated freFor aliquotation per 96 samples, make aliquotes 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.
- Preparation of the **Lysis Working Solution**

  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 Working Solution to compensate for pipetting inaccuracy especially when using multichannel pipettes etc. Use the Lysis Working Solution immediately after preparation.
- Immediately before use, resuspend **MagSi-MSP** beads by vortexing for 20 seconds.
- Prepare **Magnetic Beads / Binding Mix**:

  Per 400 µL **Binding Buffer U1** add 20 µL of **MagSi-MSP** beads. Prepare a little more Magnetic Beads / Binding Mix than needed due to loss during pipetting (e.g. for 576 extractions prepare solution for 600 extractions.
- 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 www.magtivio.com under each magtivio kit and kit component.

Infectious potential of liquid waste left over after using MagSi-NA Pathogens MSP 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, but the instrument protocol shall be adjusted for this.

6. The Elution Buffer includes 10 mM Tris pH 8.0. It does not contain EDTA. If preferred, molecular biology grade water can be used for elution.

7. Avoid samples containing precipitates, as this may result in poor results or quality. Centrifuge samples before use.

8. 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 Appropriate use of internal controls

Purified samples from MagSi-NA Pathogens MSP include nucleic acids from up to 6 samples. During the pooling process, internal controls used to monitor the extraction success and potential PCR inhibitors are also collected into a single sample. For this reason, subsequent PCR assays cannot distinguish between internal controls from the 6 samples. It is therefor recommended to apply a different strategy for internal controls. A detailed description can be found in the technical note, available at www.magtivio.com or on request by email to support@magtivio.com.

3.6 Magnetic Particle Processors

MagSi-NA Pathogens MSP has been designed for use on PurePrep 96 and allows simultaneous automated processing of up to 576 samples in 6 deepwell plates and elution in 200 µL Elution plates.

For use with other magnetic particle processors, please contact the technical support at support@magtivio.com. Protocols and consumables are available on request.

3.7 Product use limitations

MagSi-NA Pathogens MSP 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. The product is intended for use by trained personnel. The isolated nucleic acids can be used in most genomic applications, such as PCR, qPCR.

4. Protocols

Protocols are available for 6:1, 5:1 or 3:1 sample pooling. When using 5 or 6 sample plates, the instrument protocol is divided in 2 parts due to capacity limitations. Please contact magtivio for the most recent PurePrep 96 protocol files. We provide the corresponding files for direct import to the PurePrep instrument.

4.1 Sample materials and pre-treatment procedures

Preparation of the **Lysis Working Solution**

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 Working Solution to compensate for pipetting inaccuracy especially when using multichannel pipettes etc. Use the Lysis Working Solution immediately after preparation.

Preparation of the **Magnetic Beads / Binding Mix**

Per 400 µL Binding Buffer U1 add 20 µL of MagSi-MSP beads. Prepare a little more Magnetic Beads / Binding Mix than needed due to loss during pipetting (e.g. for 576 extractions prepare solution for 600 extractions).

4.2 Consumables

Suitable plates can be purchased at magtivio (see section 2.2). We strongly recommend using only the plates which are intended to use on the PurePrep 96 nucleic acid purification system. Using unsuitable plates may result in extraction failure or instrument damage.

4.3 Protocol description

Protocols with 6:1 and 5:1 pooling require two run files on the PurePrep 96 instrument, Part A and Part B. Part A is used to bind the nucleic acids from 5 or 6 sample plates to the magnetic beads. Beads are washed once and the tip comb is unloaded in Wash Buffer I. Part B is started after removal of the sample plates from instrument and placing Wash Buffer 1 (2nd step), Wash Buffer 2 and Elution buffer plates on the instrument. Part B is used to perform the subsequent washing steps and elute nucleic acids from the beads.

The protocol with 3:1 pooling require only a single instrument run and include all steps of the extraction procedure.

4.4 Instrument loading instructions

4.4.1 Instrument loading instructions for 6:1 pooling – Part A

The first protocol is to bind the nucleic acids from 6 sample plates (instrument positions 2 to 7) to the magnetic beads. Beads are washed once in Wash Buffer I (position 8).

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96 *(reusable!)*	Empty, for loading Tip-Comb only	N/A	1
Sample Plate 1 Sample Plate 2 Sample Plate 3 Sample Plate 4 Sample Plate 5 Sample Plate 6	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution Magnetic Beads / Binding Mix	200 µL 211 µL 420 µL	2 3 4 5 6 7
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	8

4.4.2 Instrument loading instructions for 6:1 pooling – Part B

The second protocol is started after removal of the sample plates from instrument positions 2 to 7 and placing Wash Buffer I (2nd step), Wash Buffer II and Elution Buffer plates on the instrument.

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample in Wash Buffer I with Tip Comb	800 µL	8
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	4
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL	5
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	100 µL	2

The Tip-Comb is unloaded at position 4.

4.4.3 Instrument loading instructions for 5:1 pooling – Part A

The first protocol is to bind the nucleic acids from 5 sample plates (instrument positions 2 to 6) to the magnetic beads. Beads are washed once in Wash Buffer I (position 7).

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96 *(reusable!)*	Empty, for loading Tip-Comb only	N/A	1
Sample Plate 1 Sample Plate 2 Sample Plate 3 Sample Plate 4 Sample Plate 5	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution Magnetic Beads / Binding Mix	200 µL 211 µL 420 µL	2, 3, 4, 5, 6
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I 🔵	800 µL	7

4.4.4 Instrument loading instructions for 5:1 pooling – Part B

The second protocol is started after removal of the sample plates from instrument positions 2 to 6 and placing Wash Buffer 1 (2nd step), Wash Buffer 2 and Elution buffer plates on the instrument.

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample in Wash Buffer I with Tip Comb	800 µL	7
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I 🔵	800 µL	4
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II 🟡	800 µL	5
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer 🟠	100 µL	2

The Tip-Comb is unloaded at position 4.

4.4.5 Instrument loading instructions for 3:1 pooling

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position ("Plate")
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96 *(reusable!)*	Empty, for loading Tip-Comb only	N/A	1
Sample Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution Magnetic Beads / Binding Mix	200 µL 211 µL 420 µL	2
Sample Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution Magnetic Beads / Binding Mix	200 µL 211 µL 420 µL	3
Sample Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution Magnetic Beads / Binding Mix	200 µL 211 µL 420 µL	4
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	5
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	6
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL	7
Elution Plate	200 µL square-well Elution Plate for KingFisher™/PurePrep 96	Elution Buffer	100 µL	8

The Tip-Comb is unloaded at position 2.

4.5 Instructions for plate filling and running the protocol

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 **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 Working Solution** to each well of each Sample Plate.

3. Add **420 µL of Magnetic Beads / Binding Mix** to each well of each Sample Plate.

4. 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.

5. 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.

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

7. Switch on the PurePrep 96 instrument and select the protocol for the specific pooling setup (6:1, 5:1 or 3:1) from the user defined protocols

8. Load the required plates to the instrument according by turning the turntable with the arrow buttons on the side.

   Make sure that all plates are inserted in the same orientation. Place the A1 well of each plate to the A1 mark on the instruments turntable.

9. Start the protocol. When running protocols with 6:1 or 5:1 pooling, unload all sample plates and place plates according to the instructions in sections 4.4.2 or 4.4.4. Then proceed with Part B of the protocol.

   At the end of the method remove all plates from the instrument, and proceed with downstream analysis or store the plate with extracted nucleic acids.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low nucleic acid yield	Insufficient sample lysis	- Make sure that the Lysis Working Solution includes Proteinase K
	Inefficient binding to the magnetic particles	- Make sure beads are well mixed before use, in both the supplied bottle as the Magnetic Beads / Binding mix - Use correct amount of all reagents - Avoid leaving bottles open as evaporation may lead to loss of binding components
	Incomplete elution	- Avoid evaporation of Elution Buffer from the plate before running the protocol. If the liquid level is too low, beads will not be resuspended and mixed properly in the elution buffer.
Problems in downstream applications / contamination in DNA sample	Insufficient washing	- Use correct amount of all reagents
	Magnetic beads remaining in the eluate	- Place the plate with eluates in a magnetic separator again, and transfer the supernatant to a new container. High amounts of co-purified genomic DNA (e.g. for saliva samples) may cause high viscosity of the eluate and force incomplete bead separation in the elution step. Optionally use higher volume of elution buffer.

6. Bulk packages

Customized bulk packages for different sample numbers, or different pooling (e.g. 2:1 or 4:1) are available on request.

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