magtivio

Technical Note

MagSi-NA Pathogens and KingFisher Flex OneStep Lysis/Binding protocol

Product Description

MagSi-NA Pathogens allows fast and cost-effective extraction of total nucleic acids from various samples like serum/plasma or swab washes. This total nucleic acid purification kit is optimized to extract pathogen DNA and RNA from samples with the highest purity and delivering nucleic acids which are suitable for qPCR based analysis. The kit includes ready-to-use buffers, Proteinase K, Poly-A-RNA and magnetic particles. The kit can be easily automated on the KingFisher Flex magnetic particle processors (Thermo Fisher Scientific) equipped with a Deep-Well Head.

The KingFisher Flex instrument can process up to 96 samples in a single run. It uses magnetic rods that collect and transfer magnetic particles across microplates with a carousel-based design, eliminating the need for multiple pipette tips. Carefully designed rod covers prevent from cross-contamination and allow for reproducible and efficient sample mixing and magnetic particle resuspension. The instrument can be integrated with liquid handling workstations and most other lab equipment typically found in DNA / RNA extraction processes, providing a walk-away solution.

Protocol information

The current technical note describes a convenient combined one step lysis and binding procedure which reduces the manual hands on time and avoids the user invention after the lysis step.

First, 211 µL of the lysis working solution per sample (premix consisting of: 200 µL Lysis Buffer PA1, 10 µL Proteinase K and 1 µL Poly-A-RNA) is added to the pre-dispensed sample into the processing plate of the KingFisher instrument. Secondly, a mix consisting of 20 µL MagSi-PA VII magnetic beads and 400 µL of Binding Buffer U1 is added into the processing plate. Following the combined lysis and binding step, three washing steps of the magnetic beads are performed. Finally the purified DNA/RNA is eluted from the magnetic beads and can be used directly for down-stream qPCR analysis. The MagSi-NA Pathogens magnetic beads are optimized for extremely fast separation times even from sample lysates with a high viscosity. The purification time per 96 samples is approximately 25 minutes.

Table 1. Reagents and equipment

Product	Art. No.	Required number per run
MagSi-NA Pathogens (96 preps)[§]	MDKT00210096	-
MagSi-NA Pathogens (10x96 preps)[§]	MDKT00210960	-
KingFisher Flex magnetic particle processor	5400620*	-
KingFisher Flex 96 Deepwell head	24074431*	-
2 mL Deep-well Plate with square wells for KingFisher™/PurePrep 96 Instrument	MDPL00200060	4
200 µL square-well Elution Plate for KingFisher™/PurePrep 96	MDPL00190060	1
96 well Tip-Comb for KingFisher™/PurePrep 96	MDPL00210060	1

[§] bulk quantities of the kit available on request
* supplied by Thermo Fisher Scientific

User notes

- KingFisher Flex protocols are available on request (email: info@magtivio.com)
- The instrument protocol is compatible with BindIt™ 4.0 software
- For tips and advice on how to adapt the instrument protocol for software of the KingFisher 96 or MagMax Express-96 instruments, please email info@magtivio.com
- For further information about the MagSi-NA Pathogens kit, please refer to the Product Manual.
- MagSi-NA Pathogens is optimized for total NA extraction from swabs, serum / plasma and other suitable sample materials.

Importing the instrument protocol

To transfer the MagSi-NA Pathogens protocol to your KingFisher Flex instrument:

- Open the BindIt software
- Press "Connect" and select the KingFisher Flex instrument that you want to save the protocol to
- Press "Transfer..." and select the folder you want to save the protocol to, e.g. User Protocols – DNA/RNA

1. Press "Upload" and select the protocol that to import
2. Optionally choose your own name for the protocol, and press OK. The software will now transfer the protocol to your KingFisher Flex instrument
3. Press "Disconnect"

Filling the extraction plates

1. Prepare a Lysis Working Solution by adding Proteinase K and Poly-A-RNA to Lysis Buffer PA1 as following:
    - Per 200 µL Lysis Buffer PA1, add 10 µL Proteinase K (20 mg/mL), 1 µL Poly-A-RNA
    - Prepare a little more Lysis Working Solution than needed due to loss during pipetting (e.g. for 96 extractions prepare solution for 100 extractions.
2. Prepare Magnetic Beads / Binding Mix as following:
    - Per 400 µL Binding Buffer U1 add 20 µL of MagSi-PA VII beads.

Table 2. Plate filling instructions for KingFisher Flex and MagSi-NA Pathogens protocol

Plate name	Plate type	Reagent	Volume
Sample Plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution MagSi-PA VII / Binding Buffer U1 mixture	200 µL 211 µL 420 µL
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 2	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL
Wash Plate 3	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer II	800 µL
Elution Plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Elution Buffer	100 µL
Tip plate	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Empty, for loading Tip-Comb only	N/A

- Prepare a little more Magnetic Beads / Binding Mix than needed due to loss during pipetting (e.g. for 96 extractions prepare solution for 100 extractions.

3. Continue by filling the plates as described in Table 2, and steps 4 to 6:

   - Sample Plate (Sample, Lysis Working Solution, MagSi-PA VII / Binding Buffer U1)

   - Wash Plate 1 and 2 (2 plates, both with Wash Buffer I)

   - Wash Plate 3 (Wash Buffer II)

   - Elution Plate (Elution Buffer)

4. Add 211 µL Lysis Working Solution to the Sample Plate.

5. Add 200 µL sample to the Sample Plate.

6. Add 420 µL Magnetic Beads / Binding Mix to the Sample Plate

   Important note:

   Mix very well prior to adding to avoid sedimentation of the beads within the dispensing step. Steps 4 and 5 can be exchanged

7. Prepare the remaining plates for the KingFisher Flex magnetic particle separator

Executing the protocol

1. Switch on the KingFisher Flex magnetic particle processor and select the “MagSi-NA Pathogens” protocol from the User Protocols

2. Start the protocol

3. Load the plates to the instrument, following the instructions on the instrument display

4. 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

5. At the end of the method remove all plates from the instrument. Follow the instructions on the instrument display