Technical Note
MagSi-NA Pathogens and KingFisher Flex

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 is 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 Deepwell head.

First, samples are incubated in Lysis Buffer PA1 to release nucleic acids from sample matrix. Lysis is supported by Proteinase K. Following the addition of the magnetic beads and Binding Buffer U1, three washing steps 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 fast separation times from even from sample lysates with a high viscosity.

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.

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 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 serum / plasma and swabs.

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 Deepwell Plate with square wells for KingFisher™	MDPL00200060	4
200 µL square-well Elution Plate for KingFisher™	MDPL00190060	1
96 well Tip-Comb for KingFisher™	MDPL00210060	1

*supplied by Thermo Fisher Scientific

Importing the instrument protocol

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

1. Open the BindIt software
2. Press "Connect" and select the KingFisher Flex instrument that you want to save the protocol to
3. Press "Transfer..." and select the folder you want to save the protocol to, e.g. User Protocols – DNA/RNA
4. Press "Upload" and select the protocol that you want to import: "MagSi-NA Pathogens.bdz"
5. Optionally choose your own name for the protocol, and press OK. The software will now transfer the protocol to your KingFisher Flex instrument

Protocol MagSi-NA Pathogens

1. Fill the plates as described in Table 2:
    - Sample Plate
    - Wash Buffer I (2 plates)
    - Wash Buffer II
    - Elution Buffer
2. 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 extraction prepare solution for 100 extractions.
3. Add 211 µL Lysis Working Solution to the sample. Mix the samples on a plate shaker at ~1000 RPM to increase lysis efficiency
4. Prepare the remaining plates for the KingFisher Flex magnetic particle separator
5. Switch on the KingFisher Flex magnetic particle processor and select the "MagSi-NA Pathogens" protocol from the User Protocols
6. Start the protocol
7. Load the plates to the instrument, following the instructions on the instrument display
8. 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
9. Dispense MagSi-PA VII beads and Binding Buffer U1 when prompted. Return plate after dispensing and continue protocol.
10. At the end of the method remove all plates from the instrument. Follow the instructions on the instrument display

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™	Sample Lysis Working Solution Dispense after Lysis: MagSi-PA VII Binding Buffer U1	200 µL 211 µL 20 µL 400 µL
Wash Buffer I - 1	2 mL Deepwell Plate with square wells for KingFisher™	Wash Buffer I	800 µL
Wash Buffer I - 2	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

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