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Technical Note

MagSi-NA Pathogens and PurePrep 96 Nucleic Acid Purification System | 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 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 PurePrep 96 Nucleic Acid Purification System with the suitable consumables.

The PurePrep 96 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 turntable-based design, eliminating the need for multiple pipette tips. Carefully designed rod covers prevent cross-contamination and allow for reproducible and efficient sample mixing and magnetic particle resuspension.

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 PurePrep 96 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. Required reagents and equipment

Product	Art. No.	Required number per run
MagSi-NA Pathogens (96 preps) [§]	MDKT00210096	-
MagSi-NA Pathogens (10x96 preps) [§]	MDKT00210960	-
PurePrep 96 Nucleic Acid Purification Instrument	AS00001	-
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

User notes

- PurePrep 96 protocol files are available on request (email: info@magtivio.com)
- Protocol files are previously imported on the instrument but can also be easily imported to the instrument via USB drive
- For tips and advice on how to adapt the instrument protocol 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, swabs and other suitable sample materials

Importing the instrument protocol (if needed)

1. To save the MagSi-NA Pathogens protocol to your PurePrep 96 Nucleic Acid Purification System:
2. Plug in the USB drive
3. Switch on the instrument
4. From the main menu select "Settings"
5. Select "Im.&export", and "Import"

6. Select the file to be imported from the list or select all files
7. Select "Import", file(s) will be uploaded to the instrument now
8. Select "Back" two times to return to the main menu
9. Select "Manage Prog."
10. Select the protocol to create a shortcut

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).

Table 2. Plate filling instructions for PurePrep 96 Nucleic Acid Purification System and MagSi-NA Pathogens protocol

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	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Sample Lysis Working Solution	200 µL 211 µL	2
		MagSi-PA VII / Binding Buffer U1 mixture	420 µL	
Wash Plate 1	2 mL Deepwell Plate with square wells for KingFisher™/PurePrep 96	Wash Buffer I	800 µL	3
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	8

2. Prepare Magnetic Beads / Binding Mix as following:
 - Per 400 µL Binding Buffer U1 add 20 µL of MagSi-PA VII beads
 - 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 200 µL sample to the Sample Plate.
5. Add 211 µL Lysis Working Solution 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 PurePrep 96 instrument

Executing the protocol

1. Load all plates to the PurePrep 96 instrument on indicated positions, Table 2

Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions
2. 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
3. Press on the Tab "Run Prog.", select the shortcut icon for the protocol and press Run to start the protocol
4. At the end of the run remove all plates from the instrument