Instructions For Use

MagSi-DX Pathogen Plus

CE

REF MDDX00030096
MDDX00030960

Σ 96 Preps
960 Preps

IVD For *in-vitro* diagnostic use

Rev. 1
23-05-2025

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Revision history		
Revision	**Release date**	**Changes**
1	23-05-2025	Initial release

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1. Components

1.1. Kit contents

MagSi-DX Pathogen Plus		
Component Name	Pack size: 96 Preps **REF:** MDDX00030096	Pack size: 960 Preps **REF.:** MDDX00030960
Lysis Buffer PA	20 mL	200 mL
Binding Buffer PA	40 mL	400 mL
Wash Buffer PA1	2 x 80 mL	2 x 800 mL
Wash Buffer PA2	80 mL	800 mL
Elution Buffer PA	20 mL	200 mL
MagSi-PA	2 mL	20 mL
Proteinase K	1.1 mL	10 x 1.1 mL
Poly-A-RNA	0.3 mg	3 mg
Product Manual	1	1

1.2. Materials to be supplied by user

Reagents:

- Nuclease-free water for reconstitution of Poly-A-RNA

Equipment:

- Magnetic separator:

Product	REF	Quantity
MM-Separator 96 Deepwell	MDMG0013	1 Unit

- Micropipettes, single and 8 or 12 channel (10-100 µL and 100-1000 µL)
- Thermoshaker, ≥1500 RPM, ≥70 °C (suggested: ThermoMixer C, Eppendorf)

Consumables:

- 96 well microplates with U-bottom and square wells (2 mL) for sample processing (Suggested: Riplate®SW 96, PP, 2 mL, Ritter, 43001-0020)
- 96 well microplates with U-bottom (300 µL) for transfer of the processed sample (Suggested: Nunc 96-Well Microplates, Round, Nonsterile, Thermo Scientific, 267245)
- Pipette tips (aerosol barrier and nuclease-free are recommended)

The required equipment for automated use may vary depending on method of processing and instrument setup or configuration. Please refer to the automated system manufacturer regarding platform-specific consumables. Additionally, refer to section 2.7 for further details regarding the automation of MagSi-DX Pathogen Plus.

1.3. About these Instructions For Use

It is recommended to thoroughly read these instruction for use before using the product.

2. Product description

2.1. Intended Purpose

MagSi-DX Pathogen Plus intended for pathogen DNA or RNA extraction from a range of clinical human samples. The purified nucleic acids can be used as an analyte for subsequent diagnostic tests.

MagSi-DX Pathogen Plus is intended for use by professional users, such as technicians and physicians, who are experienced and trained in molecular biological techniques, including the handling of potentially infectious, human sample materials.

MagSi-DX Pathogen Plus does not provide a diagnostic result. It is the sole responsibility of the user to validate the kit in conjunction with a downstream in vitro diagnostic assay, depending on the target pathogen, and to use suitable controls (e.g., internal controls, extraction controls, positive/negative control samples). Any diagnostic results generated using nucleic acids isolated with MagSi-DX Pathogen Plus in conjunction with an in-vitro diagnostic assay should be interpreted with regard to additional clinical and laboratory findings.

MagSi-DX Pathogen Plus is specifically validated for extraction of viral and bacterial nucleic acids from whole blood, serum, plasma, urine, faeces, swabs and saliva. The kit is designed to be used with any downstream application involving the amplification and detection of nucleic acids, in particular Real-Time PCR and sequencing.

The kit is designed and validated for manual use with general laboratory equipment and consumables for magnetic beads handling, but the extraction procedure can be automated using magnetic particle processors or liquid handling workstations.

2.2. Product specifications

Parameter	MagSi-DX Pathogen Plus
Technology	Magnetic beads and choatropic nucleic acid binding chemistry
Sample material	Clinical human samples
Sample volume	200 µL
Elution volume	100 µL
Processing time (manual)	approximately 60 min per 96 samples
Processing method	Manual or automated

2.3. Limitations of use

Following the instructions for use is required for nucleic acid purification. Good laboratory practices are crucial for the performance of the product. Appropriate handling of the reagents is essential to avoid contamination or impurities.

MagSi-DX Pathogen Plus is intended for extraction of pathogen nucleic acids from a range of human sample materials. The functionality of the kit has been validated using analytical panels and control samples, human samples spiked with viral DNA and RNA fragments, and human samples spiked with a gram-positive bacterial culture. The kit has not been validated for fungi or parasites.

Clinical performance of the kit in a specific diagnostic workflow must be validated by the user.

2.4. Principle of operation

The nucleic acid extraction principle is based on the release of nucleic acids and subsequent reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. Sample lysis is achieved by incubation with Proteinase K and Lysis Buffer PA containing chaotropic salts and detergents to denature and digest matrix components and to release nucleic acids from pathogens. Poly-A-RNA acts as a shield and prevents RNA degradation by protecting from RNase activity. For binding of nucleic acids to the magnetic beads, Binding Buffer PA and MagSi-PA beads are added to the lysate. After magnetic separation, the magnetic beads are washed to remove contaminants and salts using Wash Buffer PA1 and Wash Buffer PA2. Residual ethanol from previous wash steps is removed by air-drying. Finally, highly pure nucleic acids are eluted with low salt Elution Buffer PA. The purified nucleic acids can directly be used for downstream diagnostic assays.

2.5. Quality Control

In accordance with the manufacturer's Quality Management System, each lot of kits and its components are tested against predetermined specifications to ensure consistent quality.

2.6. Sample collection, handling and storage

MagSi-DX Pathogen Plus is suitable for a wide range of clinical human samples, untreated or collected and pretreated in preservation buffer. Please refer to applicable guidelines and manufacturer instructions for collection, handling and storage of clinical samples and sample, and information regarding compatibility and pre-analytical requirements of the diagnostic assay. Samples should be thoroughly mixed before use.

2.7. Use on automated systems

MagSi-DX Pathogen Plus can be used on different automated liquid handling systems or magnetic particle processing systems. However, the performance of MagSi-DX Pathogen Plus in combination of any specific automated system must be validated by the user in conjunction with an in-vitro diagnostic assay and in combination with suitable controls for the downstream application.

2.7.1. Handling of magnetic beads

A homogeneous suspension of magnetic beads is required to ensure a correct amount of magnetic beads per sample and a consistent extraction quality. Before use, shake the bottle of beads well. In automated extraction procedures, a mixing step before aspirating the beads must be integrated.

2.7.2. Liquid handling systems

MagSi-DX Pathogen Plus can be used on liquid handling workstations in combination with the MM-Separator 96 DeepWell and square-well U-bottom 96 deepwell plates, using a suitable shaking device for resuspending and mixing magnetic beads and a microplate gripper tool to transport the sample plate to and away from the magnetic separator.

2.7.3. Shaker settings

The speed settings for the microplate shaker described in the protocol that follows were defined with a specific instrument and microplate. When first using a plate shaker for incubation steps, the speed settings have to be set carefully for each specific plate to prevent cross contamination and spillage. Setting the speed of the shaker can be done by loading a microplate with a volume of dyed water equal to the working volume during each step, and step-wise increasing the shaker speed until droplets are observed on the surface of the plate. Set the shaker speed lower again.

2.7.4. Magnetic particle processing systems

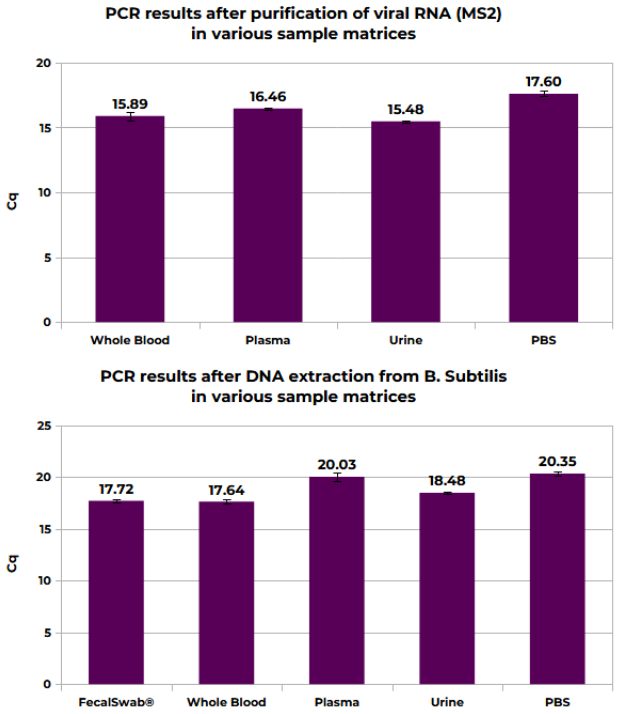
MagSi-DX Blood can be used on magnetic particle processing systems using the consumables intended for the specific system. The magnetic beads are resuspended and mixed by moving a magnetic rod cover (tip-comb) up and down, and collected by magnetic rods covered by the tip-comb. The kit components are pre-dispensed into the consumables before starting the system, and removed from the system afterwards. It is important not to exceed the maximum working volumes of the consumables.

2.8. Performance characteristics

MagSi-DX Pathogen does not have a detection, identification or measuring function and possesses no critical characteristics. Its performance is characterized by the functionality in extracting nucleic acid from various samples and has been validated in accordance with .

2.8.1. Lysis efficiency

The lysis efficiency of MagSi-DX Pathogen Plus was evaluated by spiking viral DNA and RNA as well as a culture of gram-positive bacterium (*Bacillus subtilis)* in various sample matrices. Following extraction, a PCR method targeting *B. subtilis* DNA was used to assess the effectiveness of releasing nucleic acids from difficult-to-lyse pathogens. PCR methods targeting lambda DNA and MS2 RNA were used to determine if digestive effects were also sufficient to degrade matrix components and remove these by the bind-wash-elute mechanism for diagnostic assays targeting viral pathogens. The results show that *B. subtilis* DNA was successfully extracted. Bacterial DNA, viral RNA and viral DNA could all be purified from the different sample matrices without significant differences in Cq values between sample matrices.



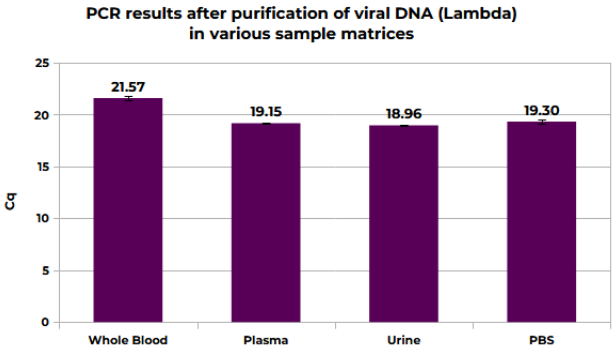


Figure 1: Comparison between qPCR results obtained after extraction from different matrices spiked with, viral RNA, viral DNA and B. subtilis. Top-left: Lambda DNA; Top-right: MS2 RNA, Bottom: B. subtilis.

2.8.2. PCR Linearity and recovery

To evaluate the PCR linearity and nucleic acid recovery of MagSi-DX Pathogen Plus, viral DNA and RNA fragments were spiked at different concentrations into PBS buffer. After extraction, the eluates were used in qPCR and RT-qPCR targeting the viral DNA and RNA fragments. Cq values were plotted against the log function of the concentration. The resulting coefficients of determination were $R^2 \geq 0.994$, indicating good linearity. Furthermore, the ΔCq values correlated with the 10-fold dilutions (ΔCq = ~3.3). The Cq's for the controls and extracted DNA/RNA corresponded well (ΔCq ≤ 0.7, data not shown) and indicate high recovery.

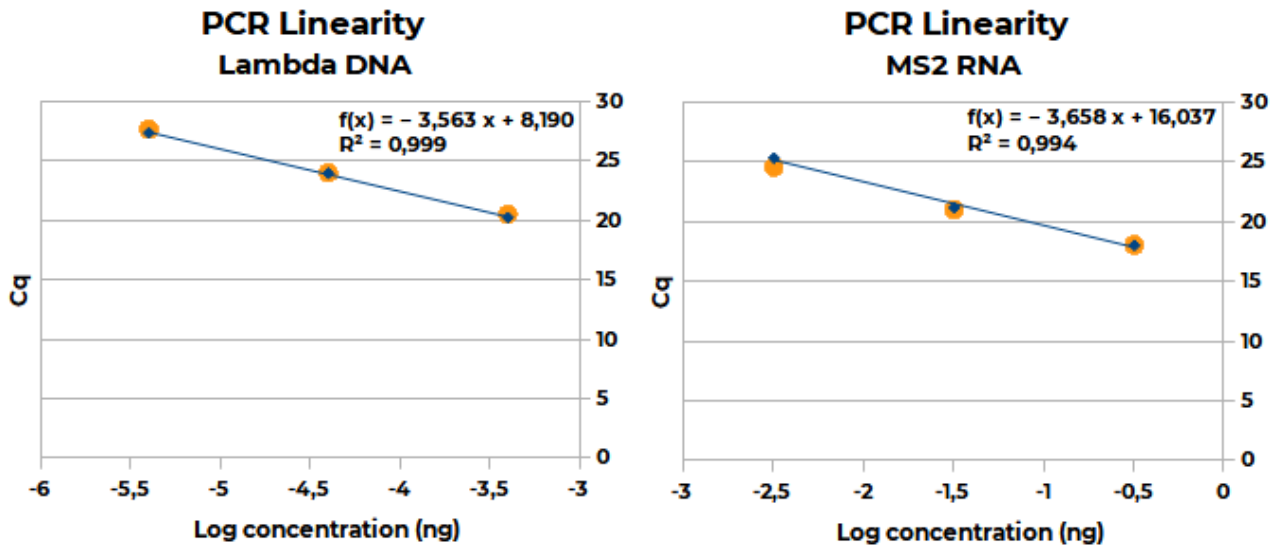


Figure 2: qPCR results obtained from 3 concentrations (10-fold dilutions) of spiked viral DNA and RNA. The blue data points represent spiked and extracted DNA and RNA. Linear regression results in $R^2 \geq 0.994$, indicating very good linearity of the extracted DNA and RNA. The orange data points represent non-extracted DNA and RNA spiked in elution buffer as controls. The Cq's for the controls and extracted DNA/RNA correspond well (ΔCq ≤ 0.7, data not shown) and indicate high recovery.

2.8.3. Matrix effects and PCR inhibition

The effect of matrix components on the performance in a diagnostic PCR test was evaluated in a spike experiment. The internal control of a Real-Time PCR kit for HCMV detection was spiked into different sample matrices. Nucleic acids were extracted with MagSi-DX Pathogen Plus and with the kit which is validated for use with the Real-Time PCR kit for HCMV. No significant differences in Cq were found for MagSi-DX Pathogen Plus, indicating absence of leftover PCR inhibitors from the samples.

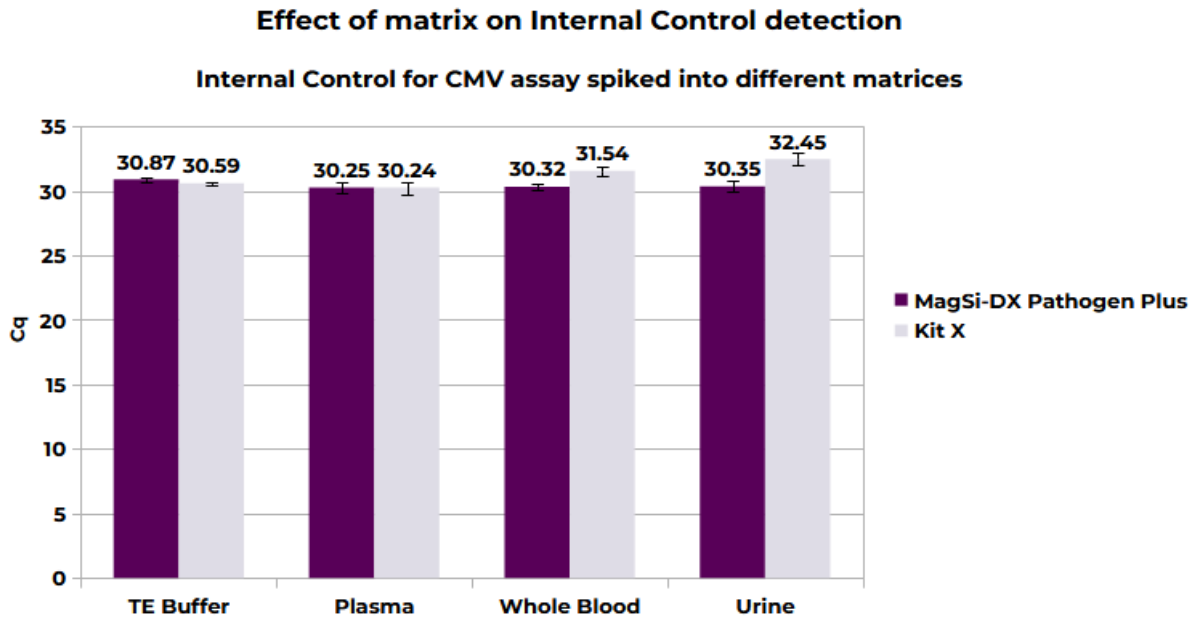


Figure 3: qPCR results obtained from spiking an internal control of a diagnostic PCR kit (CMV) in different sample matrices. Nucleic acids were extracted with MagSi-DX Pathogen Plus and a competitor kit. No significant differences in Cq were found for MagSi-DX Pathogen Plus, indicating absence of leftover PCR inhibitors from the samples.

3. Storage and Stability

All kit components, including Proteinase K, Poly-A-RNA (Lyophilized), and MagSi-PA can be stored at 18-25ºC. When stored under the conditions mentioned, the kit is stable as reported on the expiry date (see product label).

Store ready solutions of Poly-A-RNA at -20 ºC. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for 3 months.

Attention:

- Check all components included in the package for damages. If there are any damages or leakages, contact magtivio technical support and customer service.
- Do not use damaged components.
- Use RNase-free equipment.
- Do not freeze the product

3.1. Shelf life after first opening

MagSi-DX Pathogen Plus is stable for 3 months after first opening.

4. Safety Instructions

The following components of MagSi-DX Pathogen Plus contain hazardous ingredients.

Wear suitable protection clothing, gloves and goggles. Follow the safety instructions given in this section.

GHS classification

Harmful features do not need to be labeled with H and P phrases until 125 mL or 125 g.

Component	Hazardous ingredients	GHS Symbol	Hazard phrases	Precaution phrases
Lysis Buffer PA	Guanidine Hydrochloride (20-25%) CAS 50-01-1	 WARNING	H302 H319 H315	P264, P280, P302+P352 P305+P351+P338 P337+P313, P501
Binding Buffer PA	Sodium Perchlorate (50-55%) 2-Propanol (20-25%) CAS 67-63-0, 7791-07-3	 DANGER	H225 H319 H336 H373	P210, P233, P260, P280, P403+P235, P501
Wash Buffer PA1	Sodium Perchlorate (35-40%) 2-Propanol (50-55%) CAS 67-63-0, 7791-07-3	 DANGER	H225 H302 H319 H336 H373	P210, P233, P260, P280, P403+P235, P501
Wash Buffer PA2	Ethanol (70-75%) CAS 64-17-5	 DANGER	H225 H319	P210, P233, P305+P351+P338 P403+P235, P501
Proteinase K	Proteinase K (2%) CAS 39450-01-6	 DANGER	H315 H319 H334 H335	P261, P280 P284, P304+P340 P342+P311, P501

Hazard Phrases:

H225	Highly flammable liquid and vapor.
H302	Harmful if swallowed.
H302 + H332	Harmful if swallowed
H314	Causes severe skin burns and eye damage.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms, or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
H336	May cause drowsiness or dizziness.
H373	May cause damage to organs through prolonged or repeated exposure.

Precaution Phrases:

P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P284	Wear respiratory protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P303+P361+P353	IF ON SKIN (or hair): Remove contaminated clothing immediately. Rinse skin with water/shower.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses, if present and easy to do.
P310	Immediately call a POISON CENTER/doctor.
P337+P313	If eye irritation persists: Get medical advice/attention.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
P403+P235	Store in a well-ventilated place. Keep cool.
P501	Dispose of waste according to applicable legislation.

The symbol shown on the labels refer to further safety information in this section.

When working with MagSi-DX Pathogen Plus wear suitable protective clothing (e.g. lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Safety Data Sheets (available through support@magtivio.com).

CAUTION:

Lysis Buffer PA, Binding Buffer PA, and Wash Buffer PA1 contain chaotropic salt (e.g. guanidine hydrochloride and/or sodium perchlorate) which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT bring bleach in direct contact with materials exposed to the buffers mentioned. Wear suitable protective clothing, gloves, and safety goggles!

The waste generated with MagSi-DX Pathogen Plus has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to the strong denaturing lysis buffer and Proteinase K treatment, however it can not be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner, and in accordance with all local and regulatory requirements.

5. Reagent preparation

5.1. Reconstitution of Poly-A-RNA

- **MDDX00030096** (96 preps), add 120 µL nuclease-free water to the vail of Poly-A-RNA (0.3 mg) and vortex to dissolve. Store solutions of Poly-A-RNA at -20 ºC. Avoid repeated freezing and thawing. When stored accordingly, solutions are stable for at least 3 months.
- **MDDX00030960** (10x96 preps), add 1.2 mL nuclease-free water to the vail 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 ºC. Avoid repeated freezing and thawing. When stored acordingly, solutions are stable for at least 3 months.

If any precipitate is present in the buffers, warm them to 25-37 ºC to dissolve the precipitate before use.

5.2. Preparation of the Lysis Master Mix

Prepare for each sample the following Lysis Master Mix:

Component	Volume (µL)
Lysis Buffer PA	200
Poly-A-RNA (2.5 mg/mL)	1
Proteinase K solution	10
Total	211

Prepare an excess of the Lysis Master Mix to compensate for pipetting inaccuracies. Use the Lysis Master Mix immediately after preparation.

6. Protocol for Manual Nucleic Acid Extraction

Before starting:

- Prepare Lysis Master Mix according to section 5.2.
- Vortex magnetic beads thoroughly into a homogeneous suspension.
- Pre-treatment of samples (if required) according to section 2.6.

This protocol is intended for manual use of the kit. It can also be used as a guideline to set up an automated procedure on liquid handling systems.

1. Transfer **200 µL sample** to a microplate.
2. Add **211 µL Lysis Master Mix** to the microplate, and incubate at 65 ºC on a thermoshaker for 10 min with shaking at 1000 RPM.
3. Remove the sample plate from the thermoshaker and add **20 µL well-mixed MagSi-PA beads** and **400 µL Binding Buffer PA** 🟢. Place the sample plate on the plate shaker and incubate for 5 min with shaking at 1000 RPM.
4. Place the sample plate on the magnetic separator and wait 1 min to collect the beads. Remove the supernatant as completely as possible without disturbing the attracted beads pellet.
5. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer PA1** 🔵 to the samples. Resuspend the beads by placing the sample plate on the plate shaker for 1 min with shaking at 1000 RPM.
6. Place the samples on the magnetic separator and wait 1 minute to collect the beads. Remove the supernatants as completely as possible without disturbing the attracted bead pellet.
7. Repeat step 5 and 6 one time with **800 µL Wash Buffer PA1** 🔵 and one time with **800 µL Wash Buffer PA2** 🟡.
8. Remove the sample plate from the magnetic separator and dry the beads on air for 5 min to evaporate the ethanol completely.
9. Add **100 µL Elution Buffer PA** 🟠 to the sample plate. Place the sample plate on the thermoshaker and incubate for 5 min at 60 ºC with shaking at 1000 RPM.
10. Place the microplate in a magnetic separator and wait 1 min to collect the beads. Transfer the eluates to a new microplate of choice. The purified nucleic acids are ready to use for downstream diagnostic applications.

7. Control procedure

MagSi-DX Pathogen Plus does not include a control procedure. Is it the sole responsibility of the user to include suitable controls for downstream applications.

8. Interpretation of results

MagSi-DX Pathogen Plus does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream in-vitro diagnostic application depending on the target.

9. Compatibility

MagSi-DX Pathogen Plus has been validated for use with diagnostic tests based on Real-Time PCR. Please follow the manufacturer's instructions of diagnostic tests for compatibility of specific samples with a specific downstream diagnostic assay has to be validated by the user. The kit is compatible with the following samples:

- Whole blood
- Plasma
- Swabs (transport medium)
- Urine
- Feces

MagSi-DX Pathogen Plus is compatible with the following instruments for nucleic acid extraction:

- Auto-Pure 96, Auto-Pure 32A and Auto-Pure 16A (Allsheng)
- KingFisher™ Flex Purification System (Thermo Fisher Scientific)

10. Appendix

10.1. Troubleshooting

Problem	Possible cause	Comments and suggestions
Low RNA/DNA yield	RNA/DNA degradation	- Use and store the sample collection device according to manufacturer's instructions
	Inefficient binding to the magnetic particles	- Use correct amount of all reagents - Do not adjust incubation times and Increase mixing steps and incubation time for binding step - Mix sample during lysis / binding incubation
	Insufficient washing procedure	- Make sure that beads are completely resuspended in the washing steps.
	Incomplete elution	- Drying of Wash Buffer PA2 may have been incomplete, increase drying time - Completely resuspend the beads in the elution step
Problems in downstream applications / contamination in sample	Ethanol in the eluted RNA/DNA	- Increase the drying time
	Salt in the eluate	- Use buffers in the correct order - Avoid carry-over of buffers to the eluate: Make sure that all supernatants are properly removed.
	High amount of Magnetic beads remaining in the eluate	- Place the tubes with eluates in the magnetic separator again, and transfer the supernatant to a new container.

10.2. Notification requirements

Please note that any serious incident that has occurred in relation to the product shall be reported immediately to the manufacturer and the competent authority of the European member state in which the incident occurred. European vigilance contact points: https://ec.europa.eu/health/md_sector/contact_en.

10.3. Explanation of symbols

Article number

Lot number


Manufacturer


Date of manufacturing

In vitro diagnostic medical device


Consult Instructions For Use

Sufficient for <n> tests


Temperature limitation


Use by


Caution: Further information in Instruction for Use


Do not reuse

10.4. Product Use Restriction / Warranty

This product is shipped with documentation stating specifications and other technical information. magtivio warrants to meet the stated specifications. magtivio's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of magtivio, which are printed on the price list. Please contact us if you wish to get an extra copy.

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