IVD

Roche

MagNA Pure 24 Total NA Isolation Kit

Version 04

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Prefilled reagents to be used with the MagNA Pure 24 Instrument (Cat. No. 07 290 519 001) to isolate genomic DNA and viral nucleic acids from up to 1,000 µL whole blood, plasma or serum, from up to 5 mg fresh-frozen tissue, from up to 6 mm^3 formalin-fixed, paraffin-embedded tissue, or from up to 1×10^6 cultured cells, as well as for the isolation of bacterial, fungal, and viral nucleic acids from up to 1,000 µL human sample material, or human cell-free nucleic acids from up to 4,000 µL plasma.

REF 07 658 036 001 Kit for up to 96 isolations (200 µL)

Store at +15 to +25°C

⚠ Keep the kit protected from light.

⚠ Keep the kit away from magnets.

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1. INTENDED USE

The MagNA Pure 24 System is an automated nucleic acid purification system consisting of the MagNA Pure 24 instrument, software, consumables, and reagents. The MagNA Pure 24 System is intended for use by professional users for the purification of nucleic acids from biological samples for *in vitro* diagnostic purposes.

The MagNA Pure 24 Total NA Isolation Kit is for use with the MagNA Pure 24 System.

2. SUMMARY AND EXPLANATION OF THE KIT

The MagNA Pure 24 Total NA Isolation Kit is designed to isolate nucleic acids (NA) from different sample materials and different sample volumes as shown in the following table.

Target material	Sample material
Genomic DNA	▪ 200, 500 or 1,000 µL whole blood ▪ up to 1×10^6 of cultured cells ▪ up to 5 mg of fresh-frozen tissue ▪ up to 6 mm^3 formalin-fixed, paraffin-embedded tissue (FFPET)
Bacterial, fungal, or viral NA	200, 500 or 1,000 µL of plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, and urine.
Human cell-free NA	2,000 or 4,000 µL of plasma

The isolated and purified nucleic acids meet the quality standards required for highly sensitive quantitative PCR/RT-PCR analysis and next-generation sequencing.

3. PRINCIPLE OF THE PROCEDURE

The nucleic acid isolation procedure is based on the proven MagNA Pure Magnetic Glass Particle (MGP) Technology.

The key steps of this isolation procedure are:

1. Sample material is lysed, nucleic acids are released, and nucleases are denatured.
2. Nucleic acids bind to the silica surface of the added MGP due to the chaotropic salt conditions and the high ionic strength of the lysis/binding buffer.
3. MGP with bound nucleic acids are magnetically separated from the residual lysed sample.
4. Unbound substances, such as proteins, cell debris, and PCR inhibitors are removed by several washing steps.
5. Purified nucleic acids are eluted from the MGP.

4. REAGENTS

The kit is designed to perform up to 96 isolations depending on the processed sample volume.

4.1 Number of isolations

Number of isolations	Sample material
3 × 32 isolations	**Small volume:** Up to 200 µL plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, urine, up to 5 × 10^5 cultured cells, and up to 5 mg of fresh-frozen tissue.
3 × 24 isolations	**Large volume:** 500 µL or 1,000 µL of plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/ nasal swabs, stool, urine, and up to 1 × 10^6 cultured cells. Up to 6 mm^3 formalin-fixed, paraffin-embedded tissue, corresponding to 6 FFPET sections of 4 or 5 µm.
3 × 24 isolations	**Extra-large volume:** 2,000 µL or 4,000 µL of plasma. To process extra-large sample volumes, additional reagents are required.

4.2 Materials provided

The kit is comprised of 3 reagent cassettes (each with 6 reagent containers) and 12 MGP tubes. All kit components are ready-to-use.

3 Reagent cassettes	Contents/function	Composition
Reagent container 1	▪ Wash Buffer I ▪ To remove impurities.	70 mL Guanidine chloride, ethanol, Tris-HCl
Reagent container 2	▪ Proteinase K ▪ To digest proteins.	12 mL Proteinase K, glycerol
Reagent container 3	▪ Lysis Buffer ▪ For cell/pathogen lysis and binding of nucleic acids.	30 mL Guanidine thiocyanate, polydocanol, Tris-HCl

Reagent container 4	▪ Wash Buffer II ▪ To remove impurities.	34 mL Ethanol, Na-acetate
Reagent container 5	▪ Elution Buffer ▪ To elute nucleic acids.	15 mL Tris-HCl
Reagent container 6	▪ Wash Buffer III ▪ To remove impurities.	60 mL Na-acetate
12 MGP tubes	▪ Magnetic Glass Particles ▪ To bind nucleic acids.	1.9 mL Magnetic Glass Particles, isopropanol

⚠ Do not remove the individual reagent containers from the reagent cassettes. For safety symbols and warnings, refer to the corresponding safety data sheet (SDS).

Fig. 1: Example of a product image – Reagent cassette with reagent containers 1 to 6.

5. PRECAUTIONS AND HANDLING REQUIREMENTS

5.1 Warnings and precautions

- All human sourced material and all resulting waste should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4[1) 2)].
- Only personnel proficient in handling infectious materials and the use of the MagNA Pure 24 System should perform the procedures described in this Instructions for Use.
- As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Closely follow procedures and guidelines provided to ensure that the nucleic acid isolation and purification is performed correctly. Any deviation from the procedures and guidelines may affect optimal purification performance.
- Use only reagents provided in this kit and buffers recommended in the Instructions for Use. Substitutions may introduce RNases.
- Use only supplied or specified required consumables to ensure optimal nucleic acid isolation and purification performance.

5.2 Reagent handling

- Several buffers in the MagNA Pure 24 Total NA Isolation Kit contain dangerous or hazardous compounds. Do not allow reagents to touch the skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If the reagents are spilled, dilute the spill with water before wiping it up.
- Do not allow reagents containing guanidine thiocyanate (Lysis Buffer) to contact sodium hypochlorite (bleach) solution or acids. These mixtures can produce a highly toxic gas. This precaution is particularly important to be aware of when cleaning the processing station adapter, the liquid waste insert, tip waste container, and the reagent tip park. For more details regarding the cleaning maintenance, refer to the MagNA Pure 24 User Assistance.
- Before use, visually inspect the reagent cassettes to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for nucleic acid isolation and purification.
- Avoid microbial and nuclease contamination of reagents once they are opened.
- Immediately after use, cap all reagent bottles with their dedicated caps for reuse on the same instrument and store them according to their Instructions for Use.

5.3 Good laboratory practice

- Wear protective disposable gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and reagents to prevent contamination.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wash hands thoroughly after handling samples and reagents, and after removing gloves.

RNase-contaminated reagents and reaction vessels will degrade template RNA. Follow these guidelines to minimize the risk of contamination:

- Avoid touching surfaces or materials that could cause RNase carryover.
- Discard pipette tips in sealed containers to prevent airborne contamination.
- Clean, disinfect, and decontaminate work areas and instruments, including pipettes, with appropriate commercially available reagents.
- Use a work area specifically designated for RNA work. If possible, use reaction vessels and pipetters dedicated only for work with template RNA.
- If spillage occurs on the instrument, follow the cleaning instructions in the MagNA Pure 24 User Assistance.

5.4 Waste handling

- Safety Data Sheets (SDS) are available online at www.dialog.roche.com, or upon request from the local Roche office.
- Discard all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
- Wear protective disposable gloves, laboratory coats, and eye protection when discarding samples and kit reagents.
- To discard the reagents from the containers, follow the procedure below:
  1. Pierce the foil in the corner of one reagent container in the reagent cassette with a rigid plastic consumable, such as a serological pipette.
  2. Fold back the foil and discard the liquid into a designated waste container.
  3. Repeat steps 1 and 2 until all containers are empty.

6. STORAGE AND STABILITY

6.1 Kit and reagents

- The kit is shipped at ambient temperature.
- Keep the kit protected from light and away from magnets.

Reagent cassettes

- When stored at +15 to +25°C, the unopened reagent cassette is stable through the expiry date printed on the label.
- Onboard stability: reagent cassettes can be used for up to 12 hours at +15 to +25°C on the instrument deck after the first piercing.
- One reagent cassette can be used for up to 6 individual runs on the same instrument within 28 days. For storage, seal the reagent cassettes using the MagNA Pure Sealing Foil. Store the resealed reagent cassettes at +2 to +8°C in an upright position. Equilibrate the reagent cassettes at +15 to +25°C for 60 minutes before further use.

⚠ It is only possible to reuse partially used reagent cassettes on the same instrument. The software for each instrument tracks the inventory using reagent barcodes, and recognizes partially used reagent cassettes, to enable proper handling in the next run.

⚠ When reagent cassettes are not properly sealed, or are stored for longer than 28 days, evaporation may negatively affect the performance of the isolation and purification process.

⚠ When storing or carrying previously opened reagent cassettes, avoid tilting to prevent leakage.

MGP tubes

- When stored at +15 to +25°C, the MGP tubes are stable through the expiry date printed on the label. MGP tubes are **for single use only.**
- Once they have been thoroughly mixed, MGP tubes can be stored open on the instrument deck for up to 1 hour, prior to starting a run.

6.2 Specimen collection and storage of sample material

For sensitive nucleic acid detection, it is important to ensure proper storage of the samples. Sample stability is affected by elevated temperatures. Thaw frozen samples under slight agitation, for example, using a laboratory roller.

⚠ Validate storage conditions (i.e., temperature, time) for a specific sample material with regard to the individual IVD parameter.

⚠ Do not store sample material in sealed processing cartridges.

⚠ Do not use plasma or blood containing heparin since this can negatively impact the performance of the downstream application.

6.3 Storage of purified nucleic acids and eluates

To obtain optimal results, proceed immediately with the downstream application.

⚠ Do not store the eluates on the instrument deck.

⚠ Validate storage conditions (i.e., temperature, time) for eluates with regard to the individual IVD parameter.

⚠ If storing eluates in 8-tube strips, take care when removing the 8-cap strips to avoid cross-contamination. For the same reason, if the 8-tube strips need to be resealed, always use a new 8-cap strip.

If eluates were stored frozen, mix gently after thawing by pipetting up and down ten times before performing any downstream steps, such as PCR/RT-PCR or OD measurements. The mixing volume should be at least half of the eluate volume. When nucleic acids are not premixed and distributed evenly/homogenously in solution, results may not be reproducible in subsequent applications.

7. MATERIALS

7.1 Materials and devices required but not provided

Material	Descriptor	Catalog number
MagNA Pure 24 Instrument	instrument	07 290 519 001
MagNA Pure 24 Processing Cartridge	processing cartridge	07 345 577 001
MagNA Pure 24 Processing Tip Park / Piercing Tool	processing tip park / piercing tool	07 345 585 001
MagNA Pure 24 Piercing Tool	piercing tool	07 534 205 001
MagNA Pure Tip 1,000 µL	1,000 µL pipette tip	06 241 620 001
MagNA Pure Tip Waste Tray	tip waste tray	08 185 492 001
MagNA Pure Tube 2.0 mL	2.0 mL tube	07 857 551 001
MagNA Pure Sealing Foil	sealing foil	06 241 638 001
FrameStrip® with flat caps-Low Profile	8-tube strip (low) 8-cap strip	07 345 593 001
FrameStrip® with flat caps-High Profile	8-tube strip (high) 8-cap strip	07 652 275 001

- Standard laboratory equipment: Pipettes and nuclease-free, aerosol-resistant pipette tips.

7.2 Optional materials

Material	Purpose	Catalog number
MagNA Pure 24 MGP Set	For additional isolations of nucleic acids from small, large, and extra-large sample volumes.	07 806 361 001
MagNA Pure cfNA Buffer Set	For the isolation of cell-free nucleic acids from plasma samples.	07 794 398 001
MagNA Pure LC Total Nucleic Acid Isolation Kit - Lysis/Binding Buffer Refill	For external lysis protocols.	03 246 779 001
MagNA Pure Bacteria Lysis Buffer	For the isolation of bacterial, fungal, and viral nucleic acids.	04 659 180 001

Material	Purpose	Catalog number
MagNA Pure DNA Tissue Lysis Buffer	For the isolation of nucleic acids from fresh-frozen tissue.	04 805 160 001
MagNA Pure FFPET Buffer Set	For deparaffinization and lysis of formalin-fixed, paraffin embedded tissue.	08 477 144 001
Proteinase K, PCR grade Activity (+37°C) $\geq$ 0.6 U/µL	For degradation of proteins.	03 115 828 001 03 115 844 001
S.T.A.R. buffer (Stool Transport and Recovery Buffer)	For stabilization, transport, and recovery of nucleic acids in stool samples.	03 335 208 001
MagNA Lyser Instrument	For homogenizing tissue.	03 358 968 001 as of SN 40467540 03 358 976 001 as of SN 40405218
MagNA Lyser Green Beads	For homogenizing tissue.	03 358 941 001

- Phosphate buffered saline (PBS) to dilute sample material and for sample pretreatment.

8. PROCEDURES

8.1 Purification protocols

Isolate nucleic acids using different protocols, optimized for specific sample materials.

A chosen protocol must only be run with the specified sample materials. Isolation of nucleic acids from other sample types may result in inadequate performance. Improper use may lead to clumping and loss of MGP, cross-contamination of samples, or even damage to the instrument. Combine different sample materials in the same run, only if specified. Always follow the recommended pretreatment procedures.

Protocol name	Target	Sample material[1)]	Elution volume [µL][2)]
Protocols for small sample volumes			
Pathogen 200[3)]	Bacterial, fungal, and viral NA	200 µL plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, and urine. If sample volume is less than 200 µL, dilute with PBS.	50, 100
Pathogen 200 hp[3)]	Bacterial, fungal, and viral NA	200 µL plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, and urine. If sample volume is less than 200 µL, dilute with PBS.	50, 100 Recommended when a higher performance is required, for example, regarding yield and/or purity.
Fast Pathogen 200[3), 5)]	Bacterial, fungal, and viral NA	200 µL plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, and urine. If sample volume is less than 200 µL, dilute with PBS.	50, 100

Protocol name	Target	Sample material[1)]	Elution volume [µL][2)]
External Lysis Pathogen 200	Bacterial, fungal, and viral NA	450 µL lysate from 200 µL plasma, serum, whole blood. If sample volume is less than 200 µL, dilute with PBS.	50, 100
hgDNA 200	Genomic DNA	200 µL whole blood (2×10^6 white blood cells), up to 5×10^5 cultured cells, up to 5 mg of fresh-frozen tissue. If sample volume is less than 200 µL, dilute with PBS.	50, 100
hgDNA ds 200	Genomic DNA	200 µL whole blood (2×10^6 white blood cells), up to 5×10^5 cultured cells. If sample volume is less than 200 µL, dilute with PBS.	50, 100 Recommended when double-stranded DNA is required.
Fast hgDNA 200[5)]	Genomic DNA	200 µL whole blood (2×10^6 white blood cells), up to 5×10^5 cultured cells. If sample volume is less than 200 µL, dilute with PBS.	50, 100
Protocol for FFPET samples			
DNA FFPET 1000	Genomic DNA	Up to 6 FFPET sections (4 or 5 µm each)	50, 100

Protocol name	Target	Sample material[1)]	Elution volume [µL][2)]
Protocols for large sample volumes			
Pathogen 1000[3)]	Bacterial, fungal, and viral NA	500 µL or 1,000 µL plasma, serum, whole blood, bronchoalveolar lavage (BAL), nasopharyngeal/nasal swabs, stool, and urine.	50, 100
External Lysis Pathogen 500	Bacterial, fungal, and viral NA	1,450 µL lysate from 500 µL plasma, serum, whole blood. If sample volume is less than 500 µL, dilute with PBS.	50, 100
hgDNA 1000	Genomic DNA	500 µL whole blood (5×10^6 white blood cells) or 1,000 µL whole blood (1×10^7 white blood cells), up to 1×10^6 cultured cells.	100, 200 For high performance or when using cultured cells with a high DNA content, elute in 200 µL.
Protocols for extra-large sample volume			
cfNA ss 2000	Cell-free nucleic acid, predominantly single-stranded DNA.	2,000 µL plasma[4)]	50, 100
cfNA ss 4000	Cell-free nucleic acid, predominantly single-stranded DNA.	4,000 µL plasma[4)]	50, 100

Protocol name	Target	Sample material[1)]	Elution volume [µL][2)]
cfNA ds 2000	Cell-free nucleic acid, predominantly double-stranded DNA.	2,000 µL plasma[4)]	100, 150, 200 For elution of predominantly double-stranded DNA.
cfNA ds 4000	Cell-free nucleic acid, predominantly double-stranded DNA.	4,000 µL plasma[4)]	100, 150, 200 For elution of predominantly double-stranded DNA.
cfNA ds 4000 hp	Cell-free nucleic acid, predominantly double-stranded DNA.	4,000 µL plasma[4)]	60, 150 For elution of predominantly double-stranded DNA. Recommended when a higher performance is required, for example, regarding yield and/or purity.

[1)] The sample/lysate volume pipetted manually into the processing cartridges must exactly match the sample volume specified in the global run settings.

[2)] The concentration of nucleic acid in the eluate, and therefore the sensitivity of downstream applications, can be increased by choosing a low elution volume. However, the elution efficiency and the overall nucleic acid yield may be lower compared to that of using a higher elution volume.

[3)] The Pathogen protocols are designed for the isolation of bacterial, fungal, and viral nucleic acid from different sample types of human origin. These protocols can be used directly for the indicated sample volumes or the indicated volumes may be comprised of lysate.

[4)] Plasma from Roche Cell-free DNA, K2-EDTA, or Streck Cell Free DNA BCT blood collection tubes. The pretreatment with the MagNA Pure cfNA Buffer Set is mandatory.

[5)] The Fast protocols are designed for the isolation of nucleic acids from 8 samples only.

There is an Instrument Check protocol available for troubleshooting. Contact your Roche representative for more information.

8.2 Sample materials and pretreatment procedures

To obtain optimal results in downstream applications, especially in real-time RT-PCR assays, for example using the LightCycler® Instruments, do not process samples with higher volume than the selected purification protocol is designed to handle. Doing so will negatively affect the performance of the isolation and purification process, and may lead to clumping and loss of MGP, cross-contamination of samples, or even damage to the instrument.

I. Whole blood

Use fresh or frozen whole blood without any pretreatment. Ensure that the sample material is completely homogenized.

⚠ If the white blood cell count is above 1 × 10^7 blood cells/mL, dilute the whole blood with PBS prior to use to avoid clumping of MGP.

⚠ Ensure that there are no clots in anticoagulated whole blood samples.

II. Plasma/serum

Use fresh or frozen plasma or serum without any pretreatment except for the cfNA protocols.

⚠ If precipitates have formed, perform a short centrifugation step for 5 to 10 minutes at 1,900 × *g*; this centrifugation step is recommended for cfNA protocols. Use only the supernatant as sample.

III. Lysates for external lysis protocols

Whole blood, plasma, or serum mixed with MagNA Pure LC Total Nucleic Acid Isolation Kit - Lysis/Binding Buffer Refill.

Ensure that the lysis/binding buffer is equilibrated to +15 to +25°C before use.

Add 200 µL or 500 µL of whole blood, plasma, or serum to 250 µL or 950 µL MagNA Pure LC Total Nucleic Acid Isolation Kit - Lysis/Binding Buffer Refill, and mix by pipetting.

Protocol	External Lysis Pathogen 200	External Lysis Pathogen 500
Sample [µL]	200	500
Lysis /Binding Buffer [µL]	250	950
Total volume of lysate [µL]	450	1,450

Transfer the total volume of lysate to the processing cartridge.

IV. Various sample materials and lysates for the pathogen protocols.

Perform lysis of pathogens in many different sample types of human origin.

The following sample materials may be suitable for the Pathogen 200/200 hp, Fast Pathogen 200, and Pathogen 1,000 protocols:

Urine, bronchoalveolar lavage (BAL), swabs, stool, whole blood, plasma, serum, and bacterial cultures.

⚠ Due to the great variety of sample materials, no single universally applicable procedure is possible. The pretreatment for a semi-liquid sample (BAL, stool, etc.) for nucleic acid isolation depends on the type of sample material, sample viscosity, and particle type and content.

⚠ Any sample material using this sample preparation procedure in conjunction with any downstream IVD nucleic acid testing must be validated with regard to the individual IVD parameters.

⚠ Do not use 1,000 µL sample input volume for very viscous and cell-rich samples, such as stool.

Depending on the sample viscosity and particle type and content, samples may be used without any pretreatment.

Lysis Protocol using MagNA Pure Bacteria Lysis Buffer (BLB)

① **Liquefaction (optional)**

Liquefaction is recommended for very viscous sample materials and is a mandatory step for isolation of nucleic acids from BAL samples.

- Prepare a fresh DTT (dithiothreitol) stock solution (e.g., 5× conc. = 0.75%).
- Adjust final DTT concentration in sample to 0.15% by adding DTT stock solution.
- Incubate the sample while shaking at 850 rpm for 30 minutes at +37°C until it can be easily pipetted.

② **Addition of bacteria lysis buffer (BLB)**

Transfer the appropriate sample volume into a fresh 1.5 mL tube.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000	
Sample volume [µL]	100	250	500

③ Premix the appropriate volumes of BLB and Proteinase K:

Protocol	Pathogen 200/ 200 hp	Pathogen 1000	
BLB [µL]	100	250	500
Proteinase K [µL]	20	50	100
BLB/PK mixture [µL]	**120**	**300**	**600**

- Add this mixture to the 1.5 mL tube containing the sample and mix thoroughly using a vortex mixer.
- Incubate while shaking at 450 rpm for 10 minutes at +65°C.

④ **Incubation at +95°C (for difficult sample materials)**

To inactivate pathogenic organisms and to enhance cell lysis for some bacterial species in difficult sample materials such as stool samples, incubate the sample at +95°C. To prevent leakage, use screw-capped tubes.

- Incubate samples at +95°C for 10 minutes.

For isolation of RNA, omit the incubation at +95°C because this could negatively affect the integrity of the RNA.

- Chill samples on ice. Centrifuge briefly to collect the complete sample volume at the bottom of the tube.

⑤ Transfer the indicated volume of lysate to the processing cartridge.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000	
Lysate pipetted to processing cartridge [µL]	200	500	1,000

Pretreatment of stool samples

① Use a pea-sized amount of stool sample and suspend in 550 µL of PBS.

To avoid clogging the pipette tips with solid particles, centrifuge for 5 seconds at 500 × *g*.

For the isolation of viral RNA, alternatively, use a PBS/STAR Buffer mixture (1:1 mixture) to suspend stool samples. This may reduce possible inhibition in downstream applications.

② Transfer the appropriate volume of supernatant into a fresh 1.5 mL tube.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000
Supernatant [µL]	100	250

③ Premix appropriate volumes of BLB and Proteinase K:

Protocol	Pathogen 200/ 200 hp	Pathogen 1000
BLB [µL]	100	250
Proteinase K [µL]	20	50
BLB/PK mixture [µL]	**120**	300

- Add this mixture to the 1.5 mL tube containing the sample and mix thoroughly using a vortex mixer.

④ Incubate for 10 minutes at +65°C while shaking at 850 rpm, followed by 10 minutes incubation at +95°C.

⚠ For isolation of RNA, omit the incubation at +95°C because this could negatively affect the integrity of the RNA.

⑤ Transfer the indicated volume of lysate to the processing cartridge.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000
Lysate pipetted to processing cartridge [µL]	200	500

Pretreatment of swab samples

① Suspend a dry swab in an appropriate volume of BLB premixed with Proteinase K.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000	
BLB [µL]	200*	500*	1,000*
Proteinase K [µL]	20	50	100
BLB/PK mixture [µL]	**220**	**550**	**1,100**

* For swabs in transport medium, use one half of the volume of BLB with the other half made up of the sample in the transport medium; the final volume should match the volume in the table. Premix the BLB only with the total Proteinase K volume and add this mixture to the sample in the transport medium.

② Squeeze and remove the swab.

③ Mix thoroughly using a vortex mixer. Incubate the liquid sample at +65°C for 10 minutes while shaking at 450 rpm, followed by 10 minutes incubation at +95°C.

⚠ For isolation of RNA, omit incubation at +95°C because this could negatively affect the integrity of the RNA.

④ Chill samples on ice. Centrifuge briefly to collect the complete sample volume at the bottom of the tube.

⑤ Transfer the indicated volume of lysate to the processing cartridge.

Protocol	Pathogen 200/ 200 hp	Pathogen 1000
Lysate pipetted to processing cartridge [µL]	200	500 or 1,000

V. Cultured cells Use cultured cells resuspended in phosphate buffered saline (PBS) to isolate nucleic acids using the hgDNA 200 and hgDNA 1000 protocols.

① For DNA isolation from cultured cells grown in suspension, gently spin down the cultured cells for 5 minutes at 300 × *g*. If necessary, wash the cell pellet using PBS.

The cell pellet can be stored at -15 to -25°C for several weeks.

② Remove the culture media (or PBS) and resuspend cells in cold PBS by pipetting or shaking the tube until the cell pellet is resuspended.

③	Transfer the appropriate volume of suspension to the processing cartridge. ⚠ For the hgDNA 200 protocol, do not use more than 5×10^5 cells/ 200 µL. For the hgDNA 1000 protocol, do not use more than 1×10^6 cells. Any deviation may result in inadequate performance.

VI. Fresh-frozen tissue

Use up to 5 mg homogenized fresh-frozen tissue to isolate nucleic acids using the hgDNA 200 protocol.

Tissue homogenization by Proteinase K digestion	
①	Add up to 5 mg tissue sample into a 1.5 mL tube.
②	Add 180 µL MagNA Pure DNA Tissue Lysis Buffer and 20 µL Proteinase K to the tissue sample.
③	Incubate at +55°C until complete dissolution of the tissue (usually 3 hours to overnight). This homogenization method results in a high DNA yield and integrity.
④	Transfer the appropriate volume of lysate to the processing cartridge.
⑤	Lysate can be stored at -80 to -20°C if immediate purification is not desired.

Tissue homogenization using the MagNA Lyser Instrument	
①	Transfer up to 5 mg tissue sample into a MagNA Lyser Green Beads Tube.
②	Add 200 µL MagNA Pure DNA Tissue Lysis Buffer.
③	Homogenize the tissue in the MagNA Lyser Instrument for 30 to 40 seconds. If homogenization is not complete, repeat this step. For more details, refer to the MagNA Lyser Instrument Operator's Manual. This method is fast, however, due to mechanical shearing, the DNA may be partially fragmented.
④	Transfer the appropriate volume of lysate to the processing cartridge.

VII. Formalin-fixed, paraffin-embedded tissue

Use the MagNA Pure FFPET Buffer Set to purify nucleic acids from up to 6 FFPET sections of 4 or 5 µm thickness. This corresponds to a maximum amount of 6 mm^3 tissue.

⚠ Do not use more than the specified amount of FFPET sample, otherwise the performance of the nucleic acid purification process may be negatively affected. The yield and quality of the isolated nucleic acids are strongly related to tissue type, sample age, and fixation protocol used.

⚠ Never use partially used reagent bottles from manual workflows in automated workflows.

Sample and reagent preparation for the DNA FFPET 1000 protocol

① For each isolation, add up to 6 FFPET sections of 4 or 5 µm (≤6 mm^3 tissue) to the bottom of a 2.0 mL tube.

⚠ Remove excess paraffin from the FFPET block or FFPET slide prior to collecting FFPET sections.

⚠ For maximum nucleic acid recovery, the FFPET samples should be as close as possible to the bottom of the 2.0 mL tube prior to centrifugation.

② Centrifuge the 2.0 mL tube at 5,000 × *g* for 30 seconds at +15 to +25°C, to collect samples at the bottom of the tubes.

③ Load centrifuged sample tubes to the 2.0 mL adapters already inserted into the sample rack.

⚠ Insert sample tube adapters and 2.0 mL tubes into the sample rack correctly.

Load the sample rack in the sample rack slot of the instrument and continue creating the order.

④ Prepare the Deparaffinization Reagent:
Immediately before use, transfer 25 mL Deparaffinization Reagent supplied with the MagNA Pure FFPET Buffer Set to one of the empty barcoded 25 mL reagent bottles.

⑤ Load the instrument stations highlighted in the software with the required supplies.
Last, load the reagent rack with:

- Deparaffinization Reagent in a barcoded 25 mL reagent bottle
- Lysis Buffer reagent bottle(s)
- Isopropanol reagent bottle(s)
- Vortexed MGP tube(s)

⚠ Only load decapped reagent bottles and MGP tubes.

⚠ Avoid introducing foam/bubbles to the FFPET buffer set reagents. If bubbles form, they may be popped using a pipette tip.

⚠ You can only reuse partially used reagent bottles from automated workflows performed on the **same** instrument. The software for each instrument tracks the inventory using reagent barcodes, and recognizes partially used reagent bottles to enable proper handling in the next run. All reagent bottles have an onboard stability of 16 hours and are stable for 28 days after first use.

Once all supplies have been successfully verified, start the run.

⑥ When the run is finished, unload the instrument as described in the User Assistance. Immediately after use, cap all reagent bottles with their dedicated caps for reuse on the same instrument and store them according to their Instructions for Use.

⚠ Occasional translucent/colored eluates may be observed and can be used for downstream applications.

⚠ When discarding the waste, consider that the 2.0 mL sample tubes contain FFPET Buffer Set reagents.

VIII. Plasma for cell-free nucleic acids

Use the MagNA Pure cfNA Buffer Set when isolating cell-free nucleic acids (cfNA).

Before purifying cell-free nucleic acids, centrifuge the samples for 5 to 10 minutes at 1,000 to 1,900 × *g*. Avoid transferring any of the pellet.

⚠ Avoid introducing foam/bubbles during all pipetting steps.

① In a fresh tube compatible with the MagNA Pure 24 Sample Rack (round-bottom tube), place the appropriate volume of Proteinase K. Add the sample into the tube containing Proteinase K, mix gently, and incubate at +37°C for 20 minutes.

Protocol	cfNA ss 2000 cfNA ds 2000	cfNA ss 4000 cfNA ds 4000
Proteinase K [µL]	200	400
Sample volume [µL]	2,000	4,000

② According to the number of samples to be processed, prepare the cfNA buffer mix in bulk: pipette Cell-Free Nucleic Acid Enhancement Buffer (CELB) and add isopropanol (IPA) using an appropriately sized container. Cap and mix gently by inversion. The solution is stable for a maximum of 2 hours.

Protocol	cfNA ss 2000 cfNA ds 2000	cfNA ss 4000 cfNA ds 4000
CELB [µL]	1,750	3,500
IPA [µL]	300	600
cfNA buffer mix (CELB + IPA) [µL]	2,050	4,100

③ Add the appropriate amount of cfNA buffer mix to each sample: 2,000 µL cfNA buffer mix to 2,000 µL sample or 4,000 µL cfNA buffer mix to 4,000 µL sample. Mix thoroughly by dispensing and aspirating the liquid approximately 8 times to produce a homogeneous mixture.

⚠ Do not store the lysate.

If bubbles form, they may be removed by aspiration into a pipette tip held near the side of the tube just above the surface of the liquid. Alternatively, bubbles may be removed by capping tubes and centrifuging at 2,000 × *g* for 1 minute.

④ Load the tubes onto the sample rack. Load the sample rack into the instrument.

8.3 Isolation procedure

The MagNA Pure 24 Instrument is designed to simultaneously process up to 24 samples. For a detailed description of how to operate the instrument, refer to the MagNA Pure 24 User Assistance.

⚠ It is the user's responsibility to validate system performance for any procedures used in the laboratory.

⚠ When mixing primary tubes that hold samples, avoid introducing foam/bubbles before loading on the instrument. To ensure proper liquid level detection, avoid any droplets on the walls of the sample tubes.

⚠ Ensure that all sample tubes sit properly in the sample rack.

⚠ Aqueous sample material, such as nucleic acids dissolved in water or in liquids without biological buffer, may result in poor purification performance. For aqueous sample material, add 10× PBS to a final concentration of 1× PBS.

⚠ When reagent cassettes have been stored at temperatures below +15°C, equilibrate them at +15 to +25°C for at least one hour before use.

It is possible to use two reagent cassettes, of the same or different lots, within one purification run.

⚠ Ensure that all containers are completely inserted into the reagent cassette prior to placing them on the reagent loading station.

⚠ Before placing the MGP tubes on the instrument deck, vortex the individual tubes for 60 seconds. Load decapped MGP tubes carefully on the instrument deck immediately before starting the run. In case of spillage, replace MGP tubes.

⚠ All items loaded to the instrument must be decapped: tubes holding the samples, MGP tubes, internal control tubes, reagent bottles, and output consumables.

⚠ To avoid spillling of reagents, be careful when loading the reagent rack in the reagent rack slot.

For FFPET workflows: Small amounts of the Deparaffinization Reagent transferred to the sample processing tube do not affect the purification performance. Any remaining Lysis buffer will also not affect the results.

8.4 Ending a run

After the run has finished, unload the output consumables holding the eluates.

- Once a run has finished, the eluates should not remain onboard for more than 2 hours, otherwise the corresponding results are flagged.
- Once you have opened the instrument cover, cooling of eluates will stop.
- Small amounts of magnetic particles in the output tubes/tube-strips do not affect PCR and RT-PCR assays on LightCycler® Instruments or conventional thermal block cyclers. If removal of MGP is desired, place the output tubes/tube-strips on a magnetic plate before removing eluate.

- Do not exceed the onboard time limit of the reagent cassette; unload the reagent cassette carefully to avoid spillage. Seal the reagent cassette with a sealing foil. For further use, store partially used reagent cassettes at +2 to+8°C in an upright position.
- Complete the unloading task as guided by the instrument.

⚠ MGP tubes are for single use only and must be discarded after each run even if only partially used.

- Discard liquid and solid waste according to local regulations.
- Carefully inspect the instrument for any signs of spillage. If spillage occurred, clean the instrument as described in the MagNA Pure 24 User Assistance.
- Clean and decontaminate all accessories, as described in the MagNA Pure 24 Users Assistance.

⚠ Do not allow reagents containing guanidine thiocyanate (Lysis Buffer) to contact sodium hypochlorite (bleach) solution or acids. These mixtures can produce a highly toxic gas. For more details regarding cleaning and maintenance, refer to the MagNA Pure 24 User Assistance.

8.5 Quality control

⚠ Always run appropriate controls.

To control the complete process, starting from sample preparation to analysis, perform the following controls:

- Positive control using a sample material positive for the target.
- Negative control using a sample material negative for the target.
- Internal control (IC) by adding a defined amount of a control target to all samples to be purified. The IC is added prior to the purification step, co-purified and then, for example, amplified with your target of interest in the same PCR reaction. For applications that could produce false negative results, the use of an appropriate internal control is mandatory.

Internal controls

The instrument is able to automatically add an internal control (IC) to each sample during the purification run. The internal control volume is fixed to 20 µL per sample. Up to 2 different ICs can be loaded per run; only 1 IC can be added to each sample. To use this function, select the internal control in the global run settings. The appropriate amount of IC is calculated by the software and displayed in the run settings on the *Overview screen*. Add the indicated volume of internal control to a barcoded 2 mL tube, and load the tubes into the corresponding positions on the reagent rack.

Due to mechanical limitations, the required internal control volume is higher than simply multiplying the number of samples by 20 µL.

⚠ For cfNA protocols (i.e., 2,000 µL and 4,000 µL of starting sample), add the internal control manually to the prepared lysate. Note that the instrument IC function is available but not enabled.

⚠ For the FFPET protocol, no internal control can be added by the instrument.

9. LIMITATIONS AND INTERFERENCES

1. The MagNA Pure 24 Total NA Isolation Kit has been evaluated only for use in combination with the MagNA Pure 24 System.
2. Reliable results depend on proper sample collection, transport, storage, and handling procedures.
3. The MagNA Pure 24 Total NA Isolation Kit has been validated only for the sample material specified in this Instructions for Use. Purification of nucleic acids from other sample types may result in inadequate performance.
4. Use the protocols only in combination with the specified sample materials. Any deviation may result in inadequate performance.
5. Use of this product should be limited to personnel trained in nucleic acid isolation. Any IVD application using the sample preparation procedure in conjunction with any downstream IVD nucleic acid testing shall be validated with regard to the individual IVD parameters.
6. To minimize the risk of a negative impact on the results, adequate controls for downstream applications must be used.
7. Storage conditions (temperature, time) for samples, lysates, pellets of cultured cells, and eluates shall be validated with regard to the individual IVD parameter.
8. Appropriate performance characteristics have to be established by the user, in particular in conjunction with any downstream application. Any result shall be interpreted within the context of all relevant clinical and laboratory findings. As the analyte concentration can vary broadly amongst different specimen types, we recommend establishing cross-contamination performance, for example, by so-called checkerboard experiments (high positive next to negative samples) before going into routine testing.
9. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
10. The influence of interfering substances was evaluated with an increasing concentration series of the following prevalent substances: human hemoglobin, bilirubin, and lipids (evaluated with Pathogen 200 protocol).

10. SUPPLEMENTARY INFORMATION

10.1 Symbols

Symbols used in this publication and on this product:

Symbol	Description
⚠	Important note
🔍	Information note
REF	Catalog number
LOT	Batch code
	Temperature limitations (Store at)
Cont.	Quantity contained in the package
	Use by
D	Distributed by
GTIN	Global Trade Item Number
	Consult instructions for use.
	Manufacturer
CE	The kit complies with the requirements of the IVD directive 98/79/EC.
IVD	For *in vitro* diagnostic use.

10.2 Changes to previous version

- New protocol to purify NA from FFPET samples.
- New important notes added.
- Editorial changes and corrections.

11. TRADEMARKS

MAGNA PURE, MAGNA LYSER, and LIGHTCYCLER are trademarks of Roche.

Other product names and trademarks are the property of their respective owners.

12. REGULATORY DISCLAIMER

For *in vitro* diagnostic use.

13. REFERENCES

[1)] Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.

[2)] Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

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