For life science research only.
Not for use in diagnostic procedures.

MagNA Pure 96 Cellular RNA Large Volume Kit

Version: 13

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Prefilled reagents for the isolation of cellular RNA from up to 1 × 10^6 cultured cells, or up to 800 µl whole blood, or whole blood stabilized in PAXgene Blood RNA tubes, or up to 25 mg fresh-frozen tissue, or tissue stabilized in RNAlater, or from 1 - 10 µm sections from formalin-fixed, paraffin-embedded tissue, using the MagNA Pure 96 Instrument.

Cat. No. 05 467 535 001

1 kit
3 sets
3 x 96 isolations

Store the kit at +15 to +25°C.
⚠ ***Keep the kit away from magnets.***

1. General Information

1.1. Contents

Component	Label	Function / Description	Content
Tray 1	**Reagent Tray 1**		**3 trays**
Container 1	Wash Buffer I	▪ for removing impurities	1 container
Container 2	Wash Buffer II	▪ for removing impurities	1 container
Tray 2	**Reagent Tray 2**		**3 trays**
Container 1	Lysis/Binding Buffer	▪ for cell lysis and binding of RNA	1 container
Container 2	Proteinase K	▪ for digestion of proteins	1 container
Container 3	Proteinase K Incubation Buffer	▪ for digestion of proteins	1 container
Container 4	DNase Incubation Buffer	▪ for digestion of DNA	1 container
Container 5	Elution Buffer	▪ for elution of RNA	1 container
Bottle 1	Magnetic Glass Particles	▪ MGP suspension (brownish to black solution) ▪ for binding RNA	6 bottles
Bottle 2	DNase	▪ lyophilizate ▪ for digestion of DNA	6 glass vials
Bottle 3	DNase Incubation Buffer	▪ for reconstituion of DNase	3 bottles

The kit is designed to process up to 288 samples in a maximum of twelve runs. For details, see section "Isolation Protocols".

1.2. Storage and Stability

Storage Conditions (Product)

The kit components are stable at +15 to +25°C until the expiration date printed on the label.

Once opened, store the reagent trays and the MGP bottles at +15 to +25°C for up to 28 days.

Seal the reagent trays with a MagNA Pure Sealing Foil to avoid evaporation.

Storage Conditions (Working Solution)

Solution	Storage
DNase	Reconstituted DNase can be stored in a refrigerator at +2 to +8°C for up to 28 days.

Seal the cap of the bottle with parafilm after each use on the MagNA Pure 96 Instrument. Remove parafilm again when DNase is reused on the instrument.

Parafilm is not needed for sealing the caps of MGP bottles.

1.3. Additional Equipment and Reagent required

Additional equipment and reagents required to perform cellular RNA isolations with the MagNA Pure 96 Cellular RNA Large Volume Kit using the MagNA Pure 96 Instrument include:

- MagNA Pure 96 System Fluid (Internal) or (External)*
- MagNA Pure 96 Sealing Foil*
- MagNA Pure Tip 1000µL*
- MagNA Pure 96 Processing Cartridge*
- MagNA Pure 96 Output Plate*
- Standard laboratory equipment
- Pipettes and nuclease-free, aerosol-preventive tips to predispense samples into the MagNA Pure 96 Processing Cartridge: Standard length tips, or optional, extra-long tips of 10 cm length.
- Centrifuge for tubes (e.g., for PAXgene Blood RNA tubes)
- Phosphate buffered saline (PBS)
- Optional: RNA/DNA Stabilization Reagent for Blood/Bone Marrow*
- Optional, PAXgene Blood RNA tubes (Cat. No. 762165, available from PreAnalytix)
- Optional, new lids for PAXgene Blood RNA tubes (*e.g.*, flexible Vacucap Closures (16 mm), available from VWR)
- Optional, MagNA Pure RNA Tissue Lysis Buffer* (for fresh-frozen tissue applications)
- Optional, MagNA Lyser Instrument* or similar device for homogenization of fresh-frozen tissue
- Optional, MagNA Lyser Green Beads*
- Optional, MagNA Pure DNA Tissue Lysis Buffer* for FFPE tissue applications
- Optional, MagNA Pure FFPET Buffer Set* for FFPE tissue applications
- Optional, Proteinase K (solution)* for FFPE tissue applications
- Optional, Xylol, for FFPE tissue applications
- Optional, Ethanol, for FFPE tissue applications
- Vortex mixer or multiple vortex mixer
- RNAlater

1.4. Application

The MagNA Pure 96 Cellular RNA Large Volume Kit is specifically designed to isolate highly purified cellular RNA from cultured cells, or whole blood stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow, or whole blood stored in PAXgene Blood RNA tubes, or from fresh-frozen tissue, or tissue stabilized in RNAlater, or from 1 - 10 µm sections from formalin-fixed, paraffin-embedded tissue, using the MagNA Pure 96 Instrument.
Purified RNA can be used for RT-PCR with LightCycler® Instruments or standard thermal block cyclers.

The kit is designed to process up to:

- 3 × 96 isolations from up to 1 × 10^6 cultured cells.
- 3 × 96 isolations of 400 µl whole blood or a half PAXgene tube.
- 3 × 48 isolations of 800 µl whole blood or a whole PAXgene tube.
- 3 × 48 isolations from up to 25 mg fresh-frozen tissue.
- 3 × 96 isolations from up to 20 mg fresh-frozen tissue or tissue stabilized in RNAlater.
- 3 × 96 isolations from 1-10 µm sections, depending on the pretreatment from formalin-fixed or paraffin-embedded tissue.

1.5. Preparation Time

Assay Time

MagNA Pure 96 Instrument setup	5 to 10 minutes
Instrument run time	75 to 90 minutes

Additional hands-on time may be required for manual pre-isolation steps, for example, preparation of cultured cells, depending on the specific protocol.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

For optimal results in downstream procedures, particulary in real-time RT-PCR, do not process samples with higher volume or cell count than the selected purification protocol is designed to handle. Doing so can affect the performance of the isolation process, may lead to clumping and loss of MGPs, as well as cross-contamination of samples, or even damage to the instrument.

⚠ ***Treat all samples as potentially infectious.***

I) Cultured Cells

ⓘ *Cell pellets can be stored at -15 to -25°C for several weeks.*

ⓘ *Cultured cells resuspended in 200 µl PBS.*

⚠ ***Never use more sample material than this kit is designed to handle, for example, do not use more than 1 × 10^6 cells. Doing so will negatively affect the performance of the isolation process.***

II) PAXgene Blood RNA Tubes

Pellet of a whole PAXgene tube in 400 µl PBS (or optionally, only half of that suspension). The PAXgene Blood RNA tube is designed to hold 2.5 ml whole blood.

III) Whole Blood

ⓘ *The stabilized whole blood can be stored for 2 days at +15 to +25°C or for one month at -80°C.*

IV) Fresh-frozen tissue

Up to 25 mg fresh-frozen tissue samples, for example, liver, kidney, lung, muscle, tail of mammalian species can be used after homogenization.

ⓘ *For some tissue samples, that is, so-called "easy-to-lyse" tissues, more than the specified amount of homogenized tissue can be used, for example, brain.*

ⓘ *When purifying 25 mg fresh-frozen tissue samples with high amounts of nucleic acids, for example, spleen, an additional DNase digestion step may be required.*

V) Tissues stabilized in RNAlater

5 mg of RNAlater stabilized tissue can be used after homogenization.

VI) FFPE tissue

1 - 10 µm sections from formalin-fixed, paraffin-embedded tissue.

ⓘ *Thickness as well as yield and quality of the isolated RNA are strongly related to type of tissue, age of sample, as well as fixation protocol used.*

Control Reactions

For control reactions, prepare the following external controls:

- **Positive control**, by using a sample material positive for the target.
- **Negative control**, by using a sample material negative for the target.
- **Extraction control**, by using phosphate buffered saline (PBS) in place of a sample.
- **Internal Control** (IC), by adding manually a defined amount of a control template to all samples to be purified.

General Considerations

- Perform sample preparation, RT-PCR setup, and the RT-PCR run in separate locations to minimize the risk of carryover contamination which may cause false positive results.
- Nuclease-contaminated reagents and reaction vessels will degrade template NA, therefore
  – Avoid touching surfaces or materials that could cause nuclease carryover.
  – Use only reagents provided in this kit, since substitutions may introduce nucleases.
  – Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
  – Use only new nuclease-free aerosol-blocking pipette tips and microcentrifuge tubes.
  – Use a work area specifically designated for RNA work. If possible, use reaction vessels and pipettes dedicated only for work with template RNA.
- Do not use a kit after its expiration date.
- Wear disposable gloves and change them frequently.
- Some buffers contain dangerous or hazardous compounds. For detailed information, see Figure 1 (Reagent Tray 1), Figure 2 (Reagent Tray 2), and the following table. Do not allow these 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 to contact sodium hypochlorite (bleach) solution or acids. These mixtures produce a highly toxic gas. This precaution is particularly important when cleaning the MagNA Pure 96 Waste Cover.
- One set of reagents (tray 1, tray 2, MGP bottles, and reconstituted DNase) can be used for up to four individual runs with the MagNA Pure 96 Instrument. Once opened, the reagents can be used for additional runs on the same MagNA Pure 96 Instrument within 28 days, with proper sealing using a MagNA Pure Sealing Foil. When Reagent Trays are not properly sealed, evaporation may occur. Inappropriate storage conditions can negatively affect the performance of the isolation process.
- When storing output plates outside the MagNA Pure 96 Instrument, or longer than 32 hours on the instrument stage, seal the plate with a sealing foil.

⚠ ***It is only possible to reuse partially used reagents on the same MagNA Pure 96 Instrument. The MagNA Pure 96 Software for each instrument tracks inventory using reagent barcodes, and recognizes partially used reagents and tip trays, handling them appropriately in the next run.***

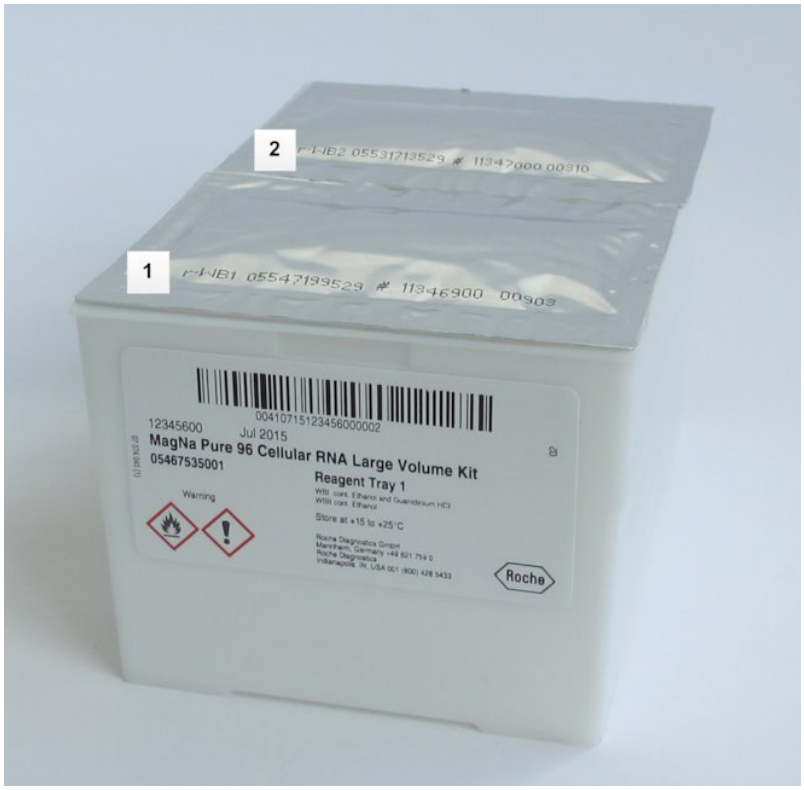

Fig. 1: Example of a product image: Reagent Tray 1

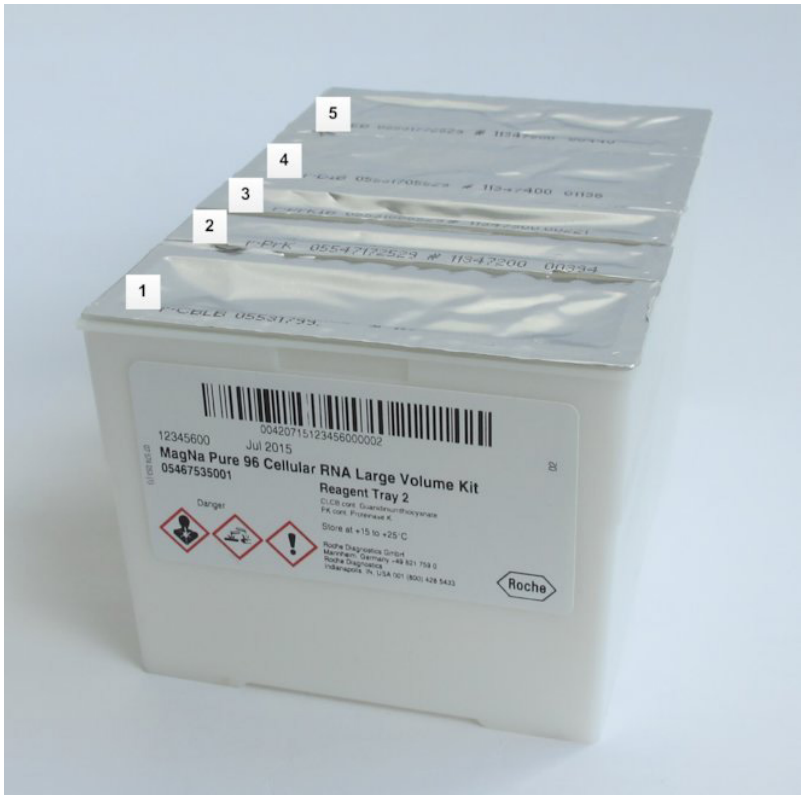

Fig. 2: Example of a product image: Reagent Tray 2

Component	Label	Dangerous/Hazardous Compounds
Tray 1	**Reagent Tray 1**	
Container 1		▪ Guanidine hydrochloride ▪ Ethanol
Container 2		▪ Ethanol
Tray 2	**Reagent Tray 2**	
Container 1	Lysis/BindingBuffer	▪ Guanidine thiocyanate
Container 2	Proteinase K	▪ Proteinase K
Container 3	Proteinase K Incubation Buffer	▪ Urea
Container 4	DNase Incubation Buffer	
Container 5	Elution Buffer	
Bottle 1	Magnetic Glass Particles	▪ Isopropanol
Bottle 2	DNase	▪ DNase
Bottle 3	DNase Incubation Buffer	

Safety Information

Precautions

Handle all samples as if potentially infectious, using safe laboratory procedures. 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.

- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

To discard reagents from the containers, follow the procedure below:

1. Pierce the foil in the corner of one container in the Reagent Tray with a solid plastic disposable such as a cell culture pipette.
2. Fold back the foil and discard the liquid in the specific container for waste.
3. Discard the contents of all containers by repeating steps 1 and 2 until all containers are empty.

Working Solution

Preparation of DNase working solution

Reagent	Preparation/Comments	Storage
DNase	For each reagent set, reconstitute **two** glass bottles of DNase (Bottle 2) with 3 ml from **one** bottle of DNase Incubation Buffer (Bottle 3). Close the bottles and mix well by inverting. Do not vortex. Once dissolved, a clear to slightly opaque solution is obtained. Transfer all liquid to the original plastic bottle labeled DNase Incubation Buffer (Bottle 3), and place a check in the box "DNase added" on the label. Close the bottle with the original lid. Mix by inverting the bottles five times.	Once reconstituted, the DNase is stable for 28 days at +2 to +8°C.

⚠ ***Reconstitute DNase just before first time use.***

Preparation of lysis solution

In an appropriate bottle or tube, premix the Lysis Buffer with Proteinase K.

(1) All volumes are for one sample.

(2) The required volume depends on the subsequent nucleic acid purification method. According to the number of samples processed, prepare the Lysis solution in bulk. Always add volume for one extra sample, and mix gently by inversion.

⚠ ***Always prepare Lysis solution immediately before each use. Avoid introducing foam or bubbles.***

Lysis Buffer [µL]	Proteinase K [µL]	Lysis solution [µL]
150	15	**165**

2.2. Protocols

Pre-Isolation steps

Cultured Cells

For cultured cells grown in suspension, gently spin down cells, for example, for 5 minutes at 300 × *g*. Wash the cell pellet using PBS. Cell pellets can be stored at -15 to -25°C for several weeks. Remove culture media (or PBS) completely. Resuspend cells in cold phosphate buffered saline (PBS) by pipetting or shaking the tube until the cell pellet is resuspended. Monolayer cultured cells should be collected using trypsinization, prior to the procedure described above. The required sample volume is 200 µl. Ensure that there are not more than 1 × 10^6 cells/200 µl.

PAXgene Tubes

For collection, storage, and transportation of whole blood in PAXgene Blood RNA tubes, follow the PAXgene instructions. The PAXgene Blood RNA tube is designed to hold 2.5 ml whole blood.
After blood sample collection, centrifuge PAXgene Blood RNA tubes in a swing-out rotor with round bottomed tube adapters at 3,000 to 5,000 × *g* for 10 minutes. Decant the supernatent as completely as possible. A reddish to brownish pellet will be clearly visible. Add 400 µl PBS to the PAXgene Blood RNA tube pellet. Close the tube using the PAXgene Blood RNA tube lid. Do not mix up lids from different samples, decant only one PAXgene Blood RNA tube at a time. Alternatively, use a new lid.
Vortex the pellet until it is resuspended completely. When using a multitube vortexer, vortex full speed for multiples of 30 seconds until the pellet is resuspended.

- When purifying RNA using the “RNA PAXgene LV” protocol, transfer the whole volume (400 µl) of the resuspended pellet from one PAXgene Blood RNA tube into one well of a MagNA Pure 96 Processing Cartridge.
- When purifying RNA using the “RNA PAXgene Half Tube LV” protocol, transfer 200 µl of the pellet resuspended in 400 µl from one PAXgene Blood RNA tube into one well of a MagNA Pure 96 Processing Cartridge.

⚠ ***Maintain PAXgene Blood RNA tubes for at least 2 hours at +15 to +25°C before starting RNA isolation.***

⚠ ***Do not use the content from more than one PAXgene Blood RNA tube per isolation to avoid clumping during the purification run.***

Whole Blood

Stabilize 400 µl (or 800 µl) whole blood with 500 µl (or 1,000 µl) RNA/DNA Stabilization Reagent for Blood/Bone Marrow*, and mix thoroughly.

Tissue homogenization for fresh-frozen tissue and tissue stabilized in RNAlater

- MagNA Lyser treatment: Transfer up to 10 mg (or up to 25 mg) tissue sample into a MagNA Lyser Green Beads Tube containing 400 µl (or 800 µl) MagNA Pure RNA Tissue Lysis Buffer.

ⓘ *For some tissue samples, so-called “easy-to-lyse” tissues, more than the specified amount of homogenized tissue can be used, for example, brain: Up to 20 mg homogenized tissue for the RNA Tissue FF Standard LV and up to 50 mg for the RNA Tissue FF High LV protocol. This requires validation by the user.*

- Avoid cooling at this step. Homogenize the tissue in the MagNA Lyser Instrument for 30 to 50 seconds (depending on the tissue type).

ⓘ *If necessary, chill on ice for 90 seconds and repeat the procedure.*

ⓘ *To minimize activity of present nucleases, perform homogenization as fast as possible. When homogenizing more than two samples, RIN values obtained using the Agilent Instrument may be lower due to prolonged time at +15 to +25°C. Tissue thawing can produce RNA degradation by RNases.*

Deparaffinization of FFPE tissue sections Using the MagNA Pure DNA Tissue Lysis Buffer

- To one 5 - 10 µm section (1 cm × 1 cm) in a 1.5 ml reaction tube, add 800 µl Xylol, incubate for 5 minutes and mix by overhead shaking.
- Add 400 µl absolute ethanol and mix. Centrifuge 2 minutes at maximum speed and discard supernatant.
- Add 1 ml absolute ethanol and mix by overhead shaking. Centrifuge for 2 minutes at maximum speed and discard supernatant.
- Invert tube and blot briefly on a paper towel to remove residual ethanol. Dry the tissue pellet for 10 minutes at +55°C.
- Add 110 µl MagNA Pure DNA Tissue Lysis Buffer.
- Add 50 µl Proteinase K solution and mix. Then incubate at +55°C until full dissolution of the tissue. The required sample volume is 150 µl.

Using the MagNA Pure FFPET Buffer Set kit

1. FFPET sample collection
   - Add 2 FFPET sections of 5 µm to a 1.5 mL tube. Cap the tube.
   - Centrifuge the tube at 5,000 x *g* for 30 seconds at +15 to +25°C, until samples collect at the bottom of the tubes. Repeat the centrifugation step if necessary.

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2. FFPET deparaffinization
   - Add 300 µL Deparaffinization Reagent directly to the FFPET sample and cap the tube.
   - Incubate while shaking at 2,000 rpm for 5 minutes at +56°C. Alternatively, incubate for 20 minutes at +56°C without shaking.

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3. Lysis solution preparation
   - Prepare the Lysis solution: In an appropriate bottle or tube, premix the Lysis Buffer with Proteinase K:

Lysis Buffer [µL]	Proteinase K [µL]	Lysis solution [µL]
150	15	**165**

   - All volumes are for one sample.
   - According to the number of samples processed, prepare the Lysis solution in bulk. Always add volume for an extra sample, and mix gently by inversion.

   ⚠ ***Always prepare Lysis solution freshly before each use.***

   ⚠ ***Avoid introducing foam or bubbles.***

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4. FFPET lysis incubation
   - Add 150 µL Lysis solution to 300 µL sample prepared in Step 2.

   i *The Lysis solution migrates to the bottom of the tube and a bilayer forms.*

   - Incubate the capped tubes at +56°C for 60 minutes.

   ⚠ ***Higher tissue input may require additional lysis incubation time.***

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5. FFPET reverse crosslinking
   - Incubate at +80°C for 30 minutes without shaking.

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6. Nucleic acid purification
   - Transfer 150 µL of the FFPET lysate from the bottom of the 1.5 mL tube.

   i *Small amounts of the Deparaffinization Reagent transferred to the sample processing tube or processing cartridge do not affect the purification performance.*

   - Immediately proceed with the nucleic acid purification.

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i Partially used reagent bottles from the pretreatment cannot be used in automated workflows performed on the MagNA Pure 24 Instrument.

Purification Protocols

Different purification protocols are available for RNA isolation with the MagNA Pure 96 Cellular RNA Kit. Each protocol is optimized for specific sample materials.

⚠ ***Run protocols only with specified sample materials, otherwise the performance of the isolation process may be negatively affected. Improper use may lead to clumping and loss of MGPs, cross-contamination of samples, or even damage to the instrument. Only the specified types of sample material can be combined in the same run.***

For each protocol, the elution volume can be chosen from the software menu.

Protocol Name	Sample Material	Elution Volume[1)]
Cellular RNA LV	Up to 1 × 10^6 cultured cells resuspended in 200 µl PBS.	50, 100, or 200 µl
RNA PAXgene LV	PAXgene pellet resuspended in 400 µl PBS.	100 or 200 µl
RNA PAXgene Half Tube LV	half PAXgene pellet contained in 200 µl PBS.	50 or 100 µl
RNA Blood LV 400	400 µl whole blood stabilized with 500 µl RNA/DNA Stabilization Reagent for Blood/Bone Marrow.	50, 100, or 200 µl
RNA Blood LV 800[3)]	800 µl whole blood stabilized with 1000 µl RNA/DNA Stabilization Reagent for Blood/Bone Marrow.	100 or 200 µl
RNA Tissue FFStandard LV	Up to 10 mg homogenized tissue from fresh-frozen or RNAlater stabilized tissue samples in a volume of 350 µl[2)].	50, 100, or 200 µl
RNA Tissue FFHigh LV[4)]	Up to 25 mg homogenized tissue from fresh-frozen tissue samples in a volume of 700 µl[2)].	100 or 200 µl
RNA Tissue FFPE LV	Deparaffinized and digested FFPE tissue sections in a volume of 150 µl.	50 or 100 µl

ⓘ *[1)] The concentration of cellular RNA in the eluate, and the sensitivity in downstream applications can be increased by choosing a lower elution volume. This may however reduce the elution efficiency, and overall RNA yield compared to using higher elution volumes. To increase total yield, use elution volumes of 100 or 200 µl.*

ⓘ *[2)] For some tissue samples, more than the specified amount of homogenized tissue can be used, for example, brain.*

ⓘ *[3)] When using the RNA Blood LV 800 protocol, the sample volume is divided to produce two samples and is processed in two separate wells of the MagNA Pure 96 Processing Cartridge. If less samples than multiples of 8 are processed using the RNA Blood LV 800 protocol, it is required to specify dummy sample data in the sample table and fill the corresponding positions in the processing cartridge with 1,800 µl PBS until the next multiple of 8 is reached.*

ⓘ *[4)] When using the RNA Tissue FF High LV protocol, the sample volume is divided to produce two samples and is processed in two separate wells of the MagNA Pure 96 Processing Cartridge.*

Isolation Procedure

General Remarks

The following procedures are used to process 96 samples at the same time. When other sample numbers than multiples of 8 are used, the instrument will process the empty positions until the next multiple of 8 is reached. For a detailed description of the instrument setup and handling, refer to the MagNA Pure 96 System Operator's Manual.

⚠ ***Ensure that kit components are equilibrated to +15 to +25°C before use. When the reagents are used at temperatures outside this recommended range, the purification may not function properly.***

⚠ ***Ensure that all containers are inserted correctly into the reagent trays, prior to placing them on the stage.***

⚠ ***Ensure that instructions are followed for type and amount of sample material (see section "Sample Material"). Using inappropriate types and amounts of sample material may cause clumping, which may lead to low yield and purity of cellular RNA, as well as cross-contamination and inhibition of downstream assays, such as in RT-PCR.***

ⓘ *To avoid erroneous volume scan results, pipette samples to the bottom of the wells of the processing cartridge while avoiding foaming and droplets on the wall of the wells.*

⚠ ***After the run has finished, carefully inspect the instrument for any signs of spillage. If spillage has occurred, clean the instrument as described in the MagNA Pure 96 System Operator's Guide.***

⚠ ***Clean and decontaminate the waste cover after each run, as described in the MagNA Pure 96 System User Training Guide. Do not use sodium hypochlorite (bleach) solution or acids for the first cleaning step, because this may produce highly toxic gas in combination with reagents containing guanidine thiocyanate.***

Procedure

For a detailed description on how to prepare and perform a purification run, refer to the MagNA Pure 96 System Operator's Manual.

Storage of RNA Eluates

⚠ ***To ensure stability of the eluted RNA, proceed immediately with RT-PCR setup. Do not store the eluted RNA on the MagNA Pure 96 Stage for a longer period of time; up to 32 hours stability on the cooled sample rack position 4 are possible.***

For storage, close the output plate with the MagNA Pure Sealing Foil and store at -15 to -25°C or -60 to -80°C. Store the RNA in aliquots if necessary, so that purified RNA is not repeatedly frozen and thawed.

ⓘ *For long-term storage, transfer eluates to an archive plate.*

⚠ ***After thawing eluates, mix gently by pipetting up and down ten times before performing any downstream steps such as RT-PCR or OD measurements. Mixing volumes should be at least half of the eluate volume. Results may not be reproducible when RNA is not premixed and distributed homogenously before pipetting.***

3. Results

High quality results with the MagNA Pure 96 Cellular RNA Large Volume Kit were demonstrated using HeLa, K-562 cultured cells, whole blood, PAXgene tubes, fresh-frozen tissues, tissue stabilized in RNAlater, and FFPE tissue sections. High yield, purity, and integrity of cellular RNA were obtained using spectrophotometry, electrophoresis, and RT-PCR.

ⓘ *Independent from the extraction method, RNA extracted from FFPE sections is often degraded due to formalin fixation.*

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads or presence of beads in the output plate.	Too much or inappropriate sample material or inefficient homogenization.	▪ Reduce sample material to values recommended in the section “Sample Material”. ▪ Use only the specified types of sample material (see section “Sample Material”).
	MGPs were magnetized prior to use.	▪ Avoid contact between MGPs and magnets prior to use. ▪ Store kit appropriately.
RNA is degraded.	Improper storage of samples.	▪ Use fresh-frozen samples, or tissue samples stabilized in RNAlater. ▪ Avoid using samples that have been stored at ambient temperature.
	Nuclease contamination	Avoid contamination of disposables and reagents with nucleases.
	Tissue homogenization was not fast enough.	Perform homogenization for fresh-frozen tissues as rapidly as possible to avoid RNase degradation. If necessary, chill on ice for 90 seconds and repeat the procedure.
Poor or no RNA yield.	Sample did not contain enough cells.	Count cells before use. For optimal number of cells, refer to section “Sample Material”.
	Storage of samples was not optimal.	▪ Use fresh-frozen samples, or tissue samples stabilized in RNAlater. ▪ Avoid using samples that have been stored at ambient temperature.
	Too much or wrong sample material.	▪ Reduce sample material to values recommended in section “Sample Material”. ▪ Use only specified types of sample material (see section “Sample Material”).
Poor RNA purity.	Too many cells in the sample.	Reduce number of cells to the values recommended in section “Sample Material” by diluting the sample.
	Drops of sample material on the walls of the wells.	Pipette samples to the bottom of each well of the processing cartridge. Avoid placing drops of sample material on the walls of the wells. Use the Sample Transfer function to automatically pipette the samples into the wells of another processing cartridge.
Poor RT-PCR performance.	Poor purity of RNA.	▪ Too much sample material used for isolation. Adjust input material to the values recommended in section “Sample Material”. ▪ Avoid sample material on the walls of wells when pipetting samples into the processing cartridge.
Black particles in the output plate.	Magnetic glass particles are present in the output plate.	Low numbers of magnetic glass particles do not affect PCR or RT-PCR assays on the LightCycler® Instrument or conventional thermal block cycler.
	RT-PCR reagents and protocols were not optimal.	Verify reagents and protocols using a positive and negative control.

Eluates show a slight color.	Drops of sample material on the walls of the wells.	Pipette samples to bottom of wells of the processing cartridge. Avoid placing drops of sample material on the walls of the wells. Use the Sample Transfer function to automatically pipette the samples into the wells of another processing cartridge.
	Too much or inappropriate sample material.	Use only the specified types of sample material (see section “Sample Material”).
Sediments in the target plate (PAXgene protocols only).	Overloading due to high blood cell content.	Centrifuge the target plate, for example for 2 minutes at 1,500 × *g*. Alternatively, use the RNA PAXgene Half Tube LV protocol. *These sediments do not affect RT-PCR assays.*

5. Additional Information on this Product

5.1. Test Principle

The MagNA Pure 96 Cellular RNA Large Volume Kit is used together with the MagNA Pure 96 Instrument to purify high-quality, intact RNA from up to 288 samples. Isolated RNA can be eluted in 50, 100, or 200 µl (depending on the Elution Volume selected in the MagNA Pure 96 Software). Purified RNA meets the quality standards required for sensitive and quantitative RT-PCR using the LightCycler® Instruments.

The RNA isolation procedure is based on the proven MagNA Pure Magnetic Glass Particle Technology. The principle steps of a MagNA Pure 96 RNA isolation procedure are:

① The sample material is lysed, nucleic acids are released, and nucleases are denatured.

② The 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 Genomic DNA is removed by incubation with DNase.

④ MGP with bound RNA are magnetically separated from the residual lysed sample.

⑤ Unbound substances, such as proteins, cell debris, and PCR inhibitors are removed by several washing steps.

⑥ Purified RNA is eluted from the MGP.

5.2. Quality Control

The kit is function tested using the following procedures: Cellular RNA is isolated from K-562 cells; the quality of the purified RNA is verified by an $OD_{260/280}$ measurement and RT-PCR using the LightCycler® 480 Instrument.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Update product name of optional reagent.
Update ordering information.
Editorial changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Consumables		
MagNA Pure 96 Processing Cartridge	36 cartridges	06 241 603 001
MagNA Pure 96 Output Plate	60 plates	06 241 611 001
MagNA Pure Tip 1000µL	40 x 96 tips	06 241 620 001
Instruments		
MagNA Pure 96 Instrument	1 instrument	06 541 089 001
Reagents, kits		
RNA/DNA Stabilization Reagent for Blood/Bone Marrow	1 bottle, 500 ml for up to 50 ml sample material	11 934 317 001
MagNA Pure 96 System Fluid (Internal)	2 containers	06 430 112 001
MagNA Pure RNA Tissue Lysis Buffer	1 bottle, 70 ml	03 604 721 001
MagNA Pure 96 System Fluid (External)	1 container	06 640 729 001
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001
MagNA Pure 96 DNA and Viral NA Small Volume Kit	For up to 3 x 192 isolations	06 543 588 001
MagNA Pure 96 DNA and Viral NA Large Volume Kit	Kit for up to 3 x 96 isolations	06 374 891 001
Proteinase K, recombinant, PCR Grade	1.25 ml, > 50 U/ml	03 115 887 001
	5 ml, > 50 U/ml	03 115 828 001
	25 ml, > 50 U/ml	03 115 844 001
MagNA Pure FFPET Buffer Set	1 kit, 2 sets To process up to 48 samples in automated workflows or up to 200 samples in manual pretreatment workflows	08 447 144 001
MagNA Pure DNA Tissue Lysis Buffer	100 mL	06 640 702 001

6.4. Trademarks

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6.5. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

6.6. Regulatory Disclaimer

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6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.
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- Safety Data Sheets
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