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

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MagNA Pure 96 Cellular RNA Large Volume Kit

Version 4.0

Content version: December 2010

Prefilled reagents for the isolation of cellular RNA from up to 1×10^6 cultured cells or whole blood stabilized in PAXgene Blood RNA tubes using the MagNA Pure 96 Instrument.

Cat. No. 05 467 535 001

Kit for 3 × 96 isolations

Store the kit at +15 to +25°C

⚠ Keep the kit away from magnets.

When properly stored, all kit components are stable until the expiration date printed on the label.

www.roche-applied-science.com

PROTOCOL

1. What this Product Does

Number of Tests

3×96 isolations from up to 1×10^6 cultured cells.
3×96 isolations of a half PAXgene tube.
3×48 isolations of a whole PAXgene tube.

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

Kit Contents

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

Storage and Stability

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

The kit is shipped at ambient temperature.

- Reagents (including reconstituted DNase) can be stored for 32 hours at +15 to +25°C on the stage of the instrument.
- Store reconstituted DNase 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.

- One set of reagent trays (tray 1 and tray 2) can be used for up to four individual runs with the MagNA Pure 96 Instrument. Once opened, the reagent trays can be used for additional runs within 28 days after proper sealing, with sealing foil and stored at +15 to +25°C.

⚠ When reagent trays are not properly sealed, or stored for longer than 28 days, evaporation may 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.

Additional Equipment and Reagents 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:

- MagNA Pure 96 System Fluid (Internal) or (External)*
- MagNA Pure 96 Sealing Foil*
- Standard laboratory equipment
- Pipettes and nuclease-free, aerosol-preventive tips (*e.g.,* extra-long tips of 10 cm length) to predispense samples into the MagNA Pure 96 Processing Cartridge
- Centrifuge for processing cartridges and for tubes (*e.g.,* for PAXgene Blood RNA tubes)
- PBS
- 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)
- Vortex mixer or multiple vortex mixer

** available from Roche Applied Science*

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 stored in PAXgene Blood RNA tubes, using the MagNA Pure 96 Instrument.

The purified cellular RNA can be used in RT-PCR on the LightCycler® Instruments or standard thermal block cyclers.

Assay Time

Setup of the MagNA Pure 96 Instrument requires approximately 5 to 10 min. Total time for the automated isolation of cellular RNA from 96 samples is approximately 75 min (85 min for isolation of cellular RNA from blood prepared in PAXgene tubes).

Additional hands-on time may be required for manual pre-isolation steps (*e.g.*, preparation of cultured cells) depending on the specific protocol. For detailed information, see section “Pre-Isolation Steps”.

2. How to Use this Product

2.1 Before You Begin

Precautions

I) Handling Requirements

- Perform sample preparation, RT-PCR setup, and the RT-PCR run in separate locations.
- Do not use a kit after its expiration date has passed.
- 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.

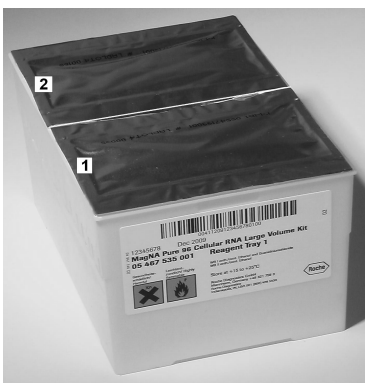

Fig. 1: Reagent Tray 1

Fig. 2: Reagent Tray 2

Component	Label	Dangerous/Hazardous Compounds
Tray 1	**Reagent Tray 1**	
Container 1	Wash Buffer I	• guanidine hydrochloride • ethanol
Container 2	Wash Buffer II	• ethanol
Tray 2	**Reagent Tray 2**	
Container 1	Lysis/Binding Buffer	• guanidine thiocyanate

Component	Label	Dangerous/Hazardous Compounds
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	

II) Laboratory Procedures

- 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.

III) Waste Handling

- Material Safety Data Sheets (MSDS) are available on the Roche Applied Science homepage (www.roche-applied-science.com), or upon request from the local Roche office.
- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling samples and kit reagents.
- To discard reagents from the containers, follow the procedure below:

❶	Pierce the foil in the corner of one container in the reagent tray with a solid plastic disposable (*e.g.*, a cell culture pipette).
❷	Fold back the foil and discard the liquid in the specific container for waste.
❸	Discard the contents of all containers by repeating steps 1 and 2 until all containers are empty.

Purification Protocols

To perform cellular RNA isolations with the MagNA Pure 96 Cellular RNA Large Volume Kit, pre-installed purification protocols are available. The elution volume must be chosen from the software menu.

Frequently check for new versions of the purification protocols.

For downloading protocols that are currently not installed, follow the procedure below:

❶	Download the protocol (*.zip* file format) from www.magnapure96.com/ (see "Download System Protocols") and save the file onto a USB stick.
❷	Log on to the MagNA Pure 96 Software as *admin* user.
❸	Insert the USB stick with the protocol into the corresponding drive.
❹	Unzip the *.zip* file.
❺	In the MagNA Pure 96 Software, select the *Utilities* tab.
❻	In the *Utilities* tab, select the *Tools* sub-tab.
❼	Click *Import Protocol*. The *Import Protocol* dialog box opens.
❽	In the *Import Protocol* dialog box, select the respective purification protocol saved on the USB stick [*Files of Type Protocols (.tdf)*].
❾	Click *Open*. The selected purification protocol is imported.
❿	Exit the MagNA Pure 96 Software and restart the control unit. Restarting the control unit is a prerequisite for using newly installed purification protocols.

For additional details, contact the local Roche representative.

⚠ Different protocols are optimized for a specific sample material.

Do not use a protocol for a sample material other than specified below. Doing so will negatively affect the performance of the isolation process and may lead to clumping and loss of MGPs, as well as cross-contamination of samples, or even damage to the instrument.

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 tube pellet contained in 200 µl	50 or 100 µl

⚠ Use latest protocol version available for purification of RNA.

⚠ Only specified types of sample material can be combined in the same run.

⚠ One set of reagent trays (tray 1 and tray 2) can be used for up to four individual runs with the MagNA Pure 96 Instrument. The pierced reagent trays can be stored for 28 days after the first use.
To prevent evaporation and contamination of reagents, the reagent trays are sealed with a sealing foil. Up to three sealing foils can be used to cover the reagent trays.

[1)] The concentration of cellular RNA in the eluate, and therefore the sensitivity in downstream applications can be increased by choosing a low elution volume; however, the elution efficiency, and therefore the overall RNA yield may be lower compared to using a higher elution volume.

Sample Material

To obtain optimal results in downstream procedures, especially in real-time RT-PCR assays on the LightCycler® Instruments, **do not process samples with higher volume or cell count than the selected purification protocol is designed to handle.** Doing so will 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 (*e.g.*, use not 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).

Reagents

The kit components (except DNase) are ready-to-use.

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

Preparation of Working Solutions

Before starting the procedure, prepare the working solutions as described below:

Reagent	Preparation/Comments	Storage
DNase	Per each reagent tray set (tray 1 and tray 2, for 96 samples), reconstitute each of **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 the bottles. Do not vortex. After complete dissolution of the lyophilizate, a clear to slightly opaque solution is obtained. Transfer all of the liquid back into 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 the DNase immediately prior to using it the first time.

Controls

To control the complete process starting from sample preparation to analysis, perform the following 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 PBS in place of a sample.

2.2 Pre-Isolation Steps

Cultured Cells

As a pre-isolation step for cellular RNA from cultured cells grown in suspension, gently spin down the cultured cells (*e.g.*, for 5 min 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. Remove the culture media (or PBS) completely and resuspend the cultured cells in cold phosphate buffered saline (PBS) by pipetting or shaking the tube until the cell pellet is resuspended completely. Monolayer cultured cells should be collected by standard trypsinization. Then follow the procedure described above. The required sample volume is 200 µl. Ensure that the number of cells per sample, 1×10^6 cells/200 µl, is not exceeded.

PAXgene Tubes

For collection, storage, and transportation of blood in PAXgene Blood RNA tubes, follow the Instructions for Use of the PAXgene supplier.

After collection of the blood sample, centrifuge the PAXgene Blood RNA tubes in a swing-out rotor with round bottomed tube adapters at 3,000 to 5,000 × *g* for 10 min. Decant the supernatent as completely as possible into a suitable waste container. A reddish to brownish pellet will be clearly visible. Add 400 µl PBS to the pellet from the PAXgene Blood RNA tube. Close the tube using the original PAXgene Blood RNA tube lid. In order not to mix up lids from different samples, decant only one PAXgene Blood RNA tube at a time. Alternatively, use a new lid available separately.

Thoroughly vortex the pellet until it is resuspended completely. When using a multitube vortexer, apply full speed vortexing for multiples of 30 sec until the pellet is resuspended completely.

- 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.

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

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

2.3 Isolation Protocols

General Remarks

The following procedures are designed to process 96 samples at the same time. When other sample numbers than multiples of 8 (SW1.1 and SW 2.0) or 24 (SW1.0) are used, the instrument will fill up the empty positions until the next multiple of 8 or 24 is reached. For a detailed description of the instrument setup and handling, refer to the MagNA Pure 96 System Quick Guide and Customer Training Guide (software version 2.0).

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

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

⚠ Ensure that the instructions are followed regarding type and amount of sample material (see section "Sample Material"). Using the wrong 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 (*e.g.,* RT-PCR).

⚠ 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 the waste cover after each run, as described in the MagNA Pure 96 System Operator´s Guide.

Procedure

For a detailed description on how to prepare and perform a purification run, refer to the MagNA Pure 96 System Quick Guide and Customer Training Guide (software version 2.0).

Storage of RNA Eluates

⚠ To ensure greatest possible 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 (although up to 32 hours stability on the MagNA Pure 96 Stage has been tested successfully).

For storage, close the output plate with the MagNA Pure 96 Sealing Foil and store at −15 to −25°C or −60 to −80°C. Store the RNA in aliquots if necessary, so that the purified RNA will not be repeatedly frozen and thawed.

⚠ After thawing eluates, mix gently by pipetting up and down ten times before performing any downstream steps, *e.g.,* RT-PCR or OD measurements. The mixing volume should be at least half of the eluate volume. When the RNA is not premixed and distributed evenly/homogenously in solution, results may not be reproducible in subsequent assays.

3. Results

Experimental Results

High quality results with the MagNA Pure 96 Cellular RNA Large Volume Kit were demonstrated using HeLa, K562 cultured cells, and PAXgene tubes. High yield, purity, and integrity of cellular RNA were detected using spectrophotometric and electrophoretic analyses, as well as sensitive and accurate RT-PCR results.

4. Troubleshooting

	Possible Cause	Recommendation
Clumping of beads or presence of beads in the output plate	Too much or wrong sample material or inefficient homogenization method.	• Reduce amount of sample material to the 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 or frozen samples. • Avoid the use of samples that have been stored extensively at ambient temperature.
	Nuclease contamination	• Avoid contamination of disposables and reagents with nucleases.
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 or frozen samples. • Avoid the use of samples that have been stored extensively at ambient temperature.
	Too much or wrong sample material.	• Reduce amount of sample material to the values recommended in section "Sample Material". • Use only the 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 dilution of the sample.
	Drops of sample material on the walls of the wells	• Pipette samples to the bottom of the wells of the 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 drops of sample material on the walls of the wells when pipetting samples into the processing cartridge.
Black particles in the output plate	Low numbers of magnetic glass particles beads 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 the RT-PCR reagents and protocols with a positive control.

	Possible Cause	Recommendation
Eluates show a slight color	Minimal abrasion from magnetic particles.	• Centrifuge the output plate at low *g*-values to remove fines. The red color does not affect RT-PCR assays on the LightCycler® Instruments or conventional thermal block cycler.
	Drops of sample material on the walls of the wells.	• Pipette samples to the bottom of the wells of the processing cartridge. • Centrifuge the processing cartridge including the samples (*e.g.*, for 2 min at 1,500 × *g*). • Use the function *Perform Sample Transfer* to automatically pipette the samples into the wells of another processing cartridge. This will ensure a transfer without the danger of contaminating the walls of the wells with sample material.
	Too much or wrong 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 (*e.g.*, for 2 min at 1,500 x *g*). Alternatively use the RNA PAXgene Half Tube LV protocol. The sediments do not affect RT-PCR assays.

5. Additional Information on this Product

How this Product Works

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 288 samples. The isolated RNA can be eluted in 50, 100, or 200 µl (depending on the Elution Volume selected in the MagNA Pure 96 Software). It meets the quality standards required for highly sensitive and quantitative RT-PCR analysis on the LightCycler® Instruments.

Test Principle

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, NA are released, and nucleases are denatured.
②	The NA bind to the silica surface of the added MGPs, 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	MGPs with bound RNA are magnetically separated from the residual lysed sample.
⑤	Unbound substances (*e.g.,* proteins, cell debris, PCR inhibitors) are removed by several washing steps.
⑥	Purified RNA is eluted from the MGPs.

Quality Control

The kit is function tested using the following procedures:

Cellular RNA is isolated from K562 cells. High quality of purified RNA is verified using agarose gel electrophoresis, $OD_{260/280}$, and RT-PCR analysis with the LightCycler® 480 Instrument.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and easy to understand, the following text conventions are used throughout these Instructions for Use:

Text Convention	Usage
Numbered stages labeled ①, ② *etc.*	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ *etc.*	Steps in a procedure that must be performed in the order listed.

Symbols In these Instructions for Use, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations In these Instructions for Use, the following abbreviations are used:

Abbreviation	Meaning
MGPs	Magnetic glass particles
PBS	Phosphate buffered saline
RT-PCR	Reverse transcription polymerase chain reaction
SW	Software

6.2 Changes to Previous Version

- Editorial changes
- Update of information linked to instrument software version 2.0

6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and guides, visit and bookmark our homepage: www.roche-applied-science.com and our Special Interest Sites including:

- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System, MagNA Pure LC System, and MagNA Pure 96 System): www.magnapure.com and www.magnapure96.com.
- Real-time PCR Systems (LightCycler® Carousel-Based System, LightCycler® 480 System, LightCycler® 1536 System, RealTime ready qPCR assays, and Universal ProbeLibrary): www.lightcycler.com.

	Product	Pack Size	Cat. No.
Instrument	MagNA Pure 96 Instrument	1 instrument, control unit and accessories	05 195 322 001
Reagents and Kits	MagNA Pure 96 Cellular RNA Small Volume Kit	3 × 192 purifications	05 467 543 001
	MagNA Pure 96 DNA and Viral NA Small Volume Kit	3 × 192 purifications	05 467 497 001
	MagNA Pure 96 DNA and Viral NA Large Volume Kit	3 × 96 purifications	05 467 454 001
	MagNA Pure 96 System Fluid (Internal)	2 Containers	05 467 098 001
	MagNA Pure 96 System Fluid (External)	1 Container	05 467 578 001
Disposables	MagNA Pure 96 Tips (1000 µl)	3840 (480 × 8)	05 392 900 001
	MagNA Pure 96 Processing Cartridge	36	05 435 315 001
	MagNA Pure 96 Sealing Foil	100	05 435 307 001
	MagNA Pure 96 Output Plate	60	05 435 285 001

6.4 Trademarks

Regulatory Disclaimer

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

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the world-wide research community.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site at:**

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.
On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

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