For general laboratory use.

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MagNA Pure Compact RNA Isolation Kit

Version 08

Content version: January 2013

Kit for isolation of total RNA from mammalian tissue, cultured cells, whole blood and blood cells

Cat. No. 04 802 993 001 Kit for 32 isolations

Store the kit at +2 to +8°C.

www.roche-applied-science.com

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PROTOCOLS

1. What this Product Does

Number of Tests 32 isolations (4 × 8) from

- up to 10 mg of mammalian tissue
- up to 1×10^6 blood cells (WBCs) or cultured cells
- up to 200 µl mammalian whole blood

Kit Contents

Vial/ Cap	Label	Contents / Function
-	Reagent Cartridge	• 32 sealed cartridges
-	Tip Tray	• 32 disposable Tip Trays • contains Reaction Tips (2 large and 1 small) and Piercing Tool
1	DNase	• 1 vial DNase solution • for digestion of residual genomic DNA
2 green cap	Lysis Buffer	• 1 bottle, 35 ml • for cell lysis or external disruption and homogenization of tissue prior to RNA isolation
-	Sample Tube	• 2 × 35 tubes, 2.0 ml • to be used for dispensing the sample; to be placed in row 1 of the Tube Rack of the MagNA Pure Compact Instrument (see Operator's Manual) • to be used for dispensing the DNase solution; to be placed in row 2 of the Tube Rack (see Operator's Manual)
-	Elution Tube	• 35 barcoded tubes, 2.0 ml • to be placed into the Elution Tube Rack of the MagNA Pure Compact Instrument (see Operator's Manual)
-	Elution Tube Cap	• 35 tube caps • to seal the Elution Tubes

Storage and Stability

- The kit components are stable at +2 to +8°C until the expiration date printed on the label.

The kit is shipped at ambient temperature.

Additional Equipment and Reagents Required

- standard laboratory equipment
- pipettes and nuclease-free, aerosol-preventive tips to dispense samples and DNase into Sample Tubes
- centrifuge and suitable nuclease-free reaction tubes
- vortex mixer
- Red Blood Cell Lysis Buffer* (optional: for pre-isolation of WBCs)
- PBS
- hemocytometer / cell counter
- homogenization device, such as:
  - MagNA Lyser Instrument* with MagNA Lyser Green Beads*
  - rotor-stator homogenizer (*e.g.,* UltraTurrax or Omni TH 220)
  - mortar/pestle/needle (0.6 mm)

** available from Roche Applied Science*

Application

Preparation of high-quality total RNA from flash-frozen mammalian tissue samples, cultured cells, whole blood, or blood cells on the MagNA Pure Compact Instrument. Unfrozen tissue, stabilized by specific reagents (*e.g.,* RNA*later*) can also be used. The purified RNA is suitable for RT-PCR on the LightCycler® Instruments and standard thermal block cyclers, as well as for other typical downstream applications in gene-expression analysis. The purified RNA is also an ideal starting material for array experiments.

⚠ For isolation of viral RNA from mammalian serum or plasma, use the MagNA Pure Compact Nucleic Isolation Kit I or the MagNA Pure Compact Nucleic Isolation Kit - Large Volume.

Assay Time

Setup of the MagNA Pure Compact Instrument requires approx. 15 min. Total time for the automated purification of RNA from 8 samples is approx. 30 min (using the 'RNA blood' protocol), 35 min (using the 'RNA cell' protocol), or 40 min (using the 'RNA tissue' protocol); no hands-on time is required after setup of the MagNA Pure Compact Instrument. Depending on the starting sample material, additional hands-on time is required for manual pre-isolation steps.

2. How to Use this Product

2.1 Before You Begin

Precautions

I) **Handling Requirements**

- Do not use a kit after its expiration date has passed.
- Some reagents contain the hazardous compounds guanidine thiocyanate (well 4, well 7 and bottle 2) or guanidine hydrochloride (well 5 and well 9). Do not let these reagents touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagents, dilute the spill with water before wiping it up.

  If you hold the Sample Cartridge with the flap pointing to the left and the two isolated wells pointing to the right, well 1 is the first well on the left side.
- Do not allow reagents containing guanidine thiocyanate (well 4, well 7 and bottle 2) to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.
- Complete each phase of the RT-PCR workflow before proceeding to the next phase. For example, you should finish RT-PCR sample preparation before starting RT-PCR setup. Sample preparation, RT-PCR setup and the RT-PCR run itself should also be performed in separate locations.

II) **Laboratory Procedures**

- Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Lysis Buffer or take appropriate measures according to local safety regulations. Roche does not warrant that samples treated with Lysis Buffer are completely inactivated and non-infectious. After sample processing is completed, remove and autoclave all disposable plastics, if you worked with potentially infectious sample material.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Do not use sharp or pointed objects when working with the reagent cartridge, in order to prevent damage of the sealing foil and loss of reagent.
- Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Regarding precautions for safe handling of RNA, see the Roche Applied Science LAB FAQS (http://www.roche-applied-science.com/labfaqs/p2_1.htm).
- Wash hands thoroughly after handling samples and test reagents.

III) **Waste Handling**

- Discard unused reagents and waste in accordance with country, federal, state and local regulations.

- Material Safety Data Sheets (MSDS) are available upon request from the local Roche office.

Purification Protocol

To perform RNA isolations with the MagNA Pure Compact RNA Isolation Kit, 3 different pre-installed purification protocols are available. For each protocol, sample and elution volumes must be chosen from the software menu.

New protocols or protocol updates can be downloaded from http:\\ www.magnapure.com. Read the instructions for downloading and installing the purification protocol carefully. For additional details, contact your local Roche representative.

All purification protocols allow the volume of Elution Buffer to be set to either 50 µl or 100 µl.

When using an elution volume of 50 µl, you can increase the concentration of RNA in the eluate and therefore, sensitivity in downstream applications. However, total RNA yield may be 20-30% lower compared to an elution volume of 100 µl.

⚠ Do not use more than 5 µl of the RNA eluate as template in a 20 µl PCR reaction.

Protocol Name	Sample Material	Procedure
RNA_Tissue	mammalian tissue (2.5 to 10 mg)	Disrupt and homogenize tissue samples manually outside the MagNA Pure Compact Instrument. After transfer of the lysates to the Tube Rack, the instrument carries out RNA purification automatically.
RNA_Cells	WBCs or cultured cells (10^3 to 10^6 cells)	Lyse cells manually outside the MagNA Pure Compact Instrument. After transfer of the lysates to the Tube Rack, the instrument carries out RNA purification automatically.
RNA_Blood	whole blood (50, 100 or 200 µl)	Set up the samples directly on the MagNA Pure Compact Tube Rack. The instrument carries out lysis and RNA purification automatically.

Sample Material

⚠ Treat all samples as potentially infectious.

To obtain optimal results in downstream procedures, especially in RT-PCR on the LightCycler® Instruments, do not process samples larger than this kit is designed to handle. Optimal amount of sample material are as follows:

- Mammalian tissue: 2.5 to 10 mg

  ⚠ For certain tissues (*e.g.*, tissues containing small cells and therefore with a high total nucleic acid content) it might be useful to start with less than 10 mg, in order to obtain an optimal RNA yield/mg tissue ratio. Due

to the high viscosity of certain tissues, using more than 10 mg of tissue might result in blocking of the MagNA Pure Compact reaction tips.

- Cultured Cells: 200 µl cell suspension containing up to 1×10^6 cultured cells.

  ⚠ The DNA content of different cell lines may vary to a large extent due to different degrees of aneuploidy. Therefore, reduce the input number of cells for cell lines with an extremely high DNA content to avoid clogging (*e.g.*, start from $2 - 5 \times 10^5$ cells).

- Mammalian whole blood: 50, 100 or 200 µl (up to 7,000 WBCs/µl).

  ⚠ Remember that different mammalian species may have different concentrations of blood cells. For some species, you may need to use a smaller sample of blood to keep the cell numbers within the above guidelines; otherwise clotting and loss of MGPs may occur which might impair the performance of the isolation process.

- WBCs: 200 µl cell suspension containing up to 1×10^6 WBCs.

  ⚠ Do not process more than 1×10^6 WBCs in a single sample. The actual concentration of WBCs in blood may differ from the values given above. If you are working with the upper limit of cell number, always count your WBCs with a hemocytometer before using them in a sample. Note that automatic counting systems (depending on the supplier) sometimes produce cell counts that do not agree with manual hemocytometer counts.

Controls

Always run appropriate controls with the samples, especially if you want to perform quantification analyses of the eluted RNA samples (*e.g.,* by LightCycler® 2.0 System RT-PCR assays). In order to control the complete process starting from sample preparation to quantification analysis, perform the following controls:

- Positive Control, by using a sample material positive for your target.
- Negative Control, by using a sample material negative for your target.
- Internal Control (IC), by analyzing an endogenous nucleic acid sequence present in all your samples.

The selection of an appropriate endogenous IC is of high importance when developing a quantitative RT-PCR assay. The IC is co-amplified with the target of interest and serves as a control for several factors: differences in initial template concentrations between different samples, sample-to-sample variations in the PCR, presence of PCR inhibitors or the extent of any RNA degradation. The advantage of using an endogenous IC is that both internal control mRNA and target mRNA are extracted from cells or tissue and reverse transcribed together. Commonly used endogenous ICs include so-called housekeeping gene mRNAs. Note that Roche Applied Science offers two LightCycler® Housekeeping Gene Sets for the detection of human housekeeping genes: G6PDH and PBGD. The level of expression of an appropriate endogenous IC should not vary with the experimental conditions or treatments to be compared.

2.2 Pre-Isolation Steps

2.2.1 General Remarks

Pre-isolation steps including manual sample lysis are required for RNA isolation from tissue, cultured cells and blood cells (WBCs).

2.2.2 Tissue

- Use only tissue samples that have been flash-frozen in liquid nitrogen immediately after excision or treated with a stabilizing agent, for example RNA*later*.
- Before adding the tissue sample to the lysis buffer, make sure that it is still completely frozen and process it immediately.
- Efficient disruption and homogenization of the sample material is essential for isolation of intracellular RNA from tissues. Incomplete tissue disruption will result in significantly reduced RNA yields.
- By homogenizing the tissue lysate, high molecular weight genomic DNA and other high molecular weight cellular components are sheared reducing the viscosity of the lysate. Incomplete homogenization will result in significantly reduced RNA yields and may cause clogging of reaction tips.
- While some disruption methods simultaneously homogenize the sample, others require an additional homogenization step.

⚠ Always prepare tissue lysates fresh and process them immediately. Tissue lysates can be stored at +2 to +8°C when RNA purification is carried out the same day, or at –70°C or below when RNA isolation is postponed for more than 1 day.

Tissue Disruption Using the MagNA Lyser Instrument

The table below describes disruption and homogenization of fresh-frozen or RNA*later*-fixed tissue using the MagNA Lyser Instrument.

❶ • Add 450 µl Tissue Lysis Buffer (bottle 2) to a MagNA Lyser Green Beads tube. Then transfer 1 to 10 mg tissue into the tube.

⊘ It is beneficial to start with (at least) double the amount of tissue and buffer for homogenization, because part of the sample lysate (bound between the homogenization beads) can not be used.

• Set up the MagNA Lyser instrument as described in the Operator's Manual.

• Start the disruption cycle applying speed and time settings appropriate for your specific sample material.

⚠ Always optimize the tissue disruption parameters (speed, time) prior to performing the actual RNA purification procedure. Insufficient disruption might lead to poor RNA yield, while excessive disruption might lead to RNA degradation. As an initial starting point, use the values given for some exemplary sample materials in the table below:

Sample Material	Speed	Time
Liver/Kidney	6,500 rpm	50 s
Spleen/Tumor Tissue RNA*later*-fixed Tissue	6,500 rpm	2 × 50 s[#]
Tail/Ear/Skin	6,500 rpm	2-3 × 50 s[#]

⚠ [#]Long disruption cycles may cause degradation of RNA by heat stress. Therefore, avoid continuous disruption cycles of more than 50 s. Rather, apply several disruption cycles of maximally 50 s. Cool the samples in the MagNA Lyser Rotor Cooling Block (included with the MagNA Lyser Instrument), or on ice, between the disruption cycles.

❷ Incubate samples 30 min (at +15 to +25°C).

❸ Centrifuge 2 min at 13,000 × *g* (at +15 to +25°C).

❹ Transfer 350 µl of the lysate supernatant into the Sample Tube.

❺ Place the Sample Tube in the Sample Rack and start the RNA_Tissue protocol as described in section 2.3.

Tissue Disruption Using a Rotor-Stator Homogenizer

The table below describes disruption and homogenization of fresh-frozen or RNA*later*-fixed tissue, using rotor-stator homogenizer (*e.g.,* UltraTurrax or Omni TH 220).

❶	Lyse and homogenize tissue with 350 µl Tissue Lysis Buffer in a rotor-stator homogenizer, following instrument supplier instructions. Depending on the type of sample, rotor-stator homogenizers thoroughly disrupt and homogenize tissue in 5 to 90 sec in the presence of Tissue Lysis Buffer. Depending on the type of tissue, several disruption cycles may be necessary. ⚠ Avoid generation of foam by keeping the tip of the homogenizer always submerged and holding the immersed tip to one side of the tube.
❷	Incubate samples 30 min (at +15 to +25°C).
❸	Centrifuge 2 min at 13,000 × *g* (at +15 to +25°C).
❹	Transfer 350 µl of the lysate supernatant into the Sample Tube.
❺	Place the Sample Tube in the Sample Rack and start the RNA_Tissue protocol as described in section 2.3.

Tissue Disruption Using Mortar/ Pestle/Syringe

The table below describes disruption and homogenization of fresh-frozen or RNA*later*-fixed tissue using rotor mortar, pestle and syringe.

❶	• Thoroughly grind 1 to 10 mg tissue in liquid nitrogen with a mortar and pestle. Transfer the frozen powder into a liquid nitrogen pre-cooled microfuge tube, that is suitable for centrifugation. • Allow the remaining liquid nitrogen to evaporate, but avoid thawing of the tissue sample. • Add 350 µl Tissue Lysis Buffer (bottle 2) to the sample, then homogenize further, by passing the sample through a syringe needle (ø 0.6 mm) several times.
❷	Incubate samples 30 min (at +15 to +25°C).
❸	Centrifuge 2 min at 13,000 × *g* (at +15 to +25°C).
❹	Transfer 350 µl of the lysate supernatant into the Sample Tube.
❺	Place the Sample Tube in the Sample Rack and start the RNA_Tissue protocol as described in section 2.3.

2.2.3 Cultured Cells

For sample preparation of up to 1 × 10^6 cultured cells follow the procedure below.

1. Pellet cells by centrifugation (10 min at 300 × *g*).
   You may flash-freeze cell pellets in liquid nitrogen and store them at -70°C for later usage.
2. Resuspend the cell pellet carefully by pipetting up and down in a suitable volume of cold (+2 to +8°C) PBS to obtain a concentration of 1 × 10^6 cells in 100 µl (check concentration, in hemocytometer / cell counter).
3. Add an equal volume of Lysis Buffer (vial 2) at +15 to +25°C and mix carefully by pipetting up and down.
4. Transfer 200 µl of the lysate into a Sample Tube.
5. Place the Sample Tube in the Sample Rack and start the "RNA_Cell" protocol as described in section 2.3.

2.2.4 Whole Blood

If you use whole blood as sample material in combination with the RNA_Blood isolation protocol, no sample preparation is necessary.

The protocol for whole blood was developed with human blood.

⚠ Use only whole blood containing anticoagulants (EDTA or Citrate). Do not use samples that were stored frozen or at ambient temperature for longer than 6 hours, because this could lead to degradation of RNA.

⚠ If you believe that your blood samples may contain a high number of blood cells (>**7,000** cells/µl), **count the cells and use correspondingly less sample to avoid overloading.**

1. Transfer 50, 100 or 200 µl whole blood directly into a Sample Tube. Make sure that the blood samples have been homogenized properly, for example, by putting them on a roller incubator for 5 to 10 min prior to transfer into the tube.
2. Place the Sample Tubes in the Tube Rack and start the "RNA_Blood" protocol as described in section 2.3.

2.2.5 White Blood Cells (WBCs)

For manual pre-isolation of up to 1×10^6 WBCs from fresh, stabilized mammalian whole blood, follow the procedure below.

❶	Warm the Red Blood Cell Lysis Buffer* to RT and chill the PBS on ice. You will need two volumes of Red Blood Cell Lysis Buffer for every volume of blood processed.
❷	• Add 1 part fresh blood to 2 parts Red Blood Cell Lysis Buffer. • Invert the sample 5 times manually.
❸	Incubate at RT for 15 min on a roller incubator, until you see a clear solution (indicating complete red blood cell lysis). **Never vortex!**
❹	Centrifuge 10 min at 700 × *g* (at +15 to +25°C).
❺	Carefully remove the supernatant with a pipette and discard.
❻	Suspend the WBC pellet in 1 ml Red Blood Cell Lysis Buffer and transfer into an Eppendorf vial.
❼	Centrifuge 3 min at 700 × *g* (at +15 to +25°C).
❽	Carefully remove the supernatant with a pipette and discard.
❾	Resuspend the cell pellet carefully by pipetting up and down in a suitable volume of cold (+2 to +8°C) PBS to obtain a concentration of 1×10^6 WBCs in 100 µl (check concentration, using a hemocytometer or cell counter).
❿	• Add an equal volume Lysis Buffer (vial 2), then mix carefully by pipetting up and down. • Incubate for 15 to 30 min at +15 to +25°C.
⓫	Transfer 200 µl of the lysate into a Sample Tube.
⓬	Place the Sample Tube in the Sample Rack and start the RNA_Cell protocol as described in section 2.3.

General Remarks

The following procedure is designed to process 8 samples at the same time. The instrument can handle all numbers of samples between 1 and 8. For a detailed description of instrument setup and handling, refer to the MagNA Pure Compact Operator's Manual.

⚠ If you use frozen sample material:

- Tissue: Thaw frozen lysates by incubating them 5 min at 37°C with occasional shaking. Vortex thoroughly, then centrifuge briefly to collect the complete lysate.
- Cells: carefully resuspend frozen cell pellets by pipetting up and down in a suitable volume of cold PBS (+2 to +8°C), to obtain a concentration of 1 x 10^6 cells in 100 µl. Then add an equal volume of Lysis Buffer and mix carefully by pipetting up and down.

⚠ Equilibrate the Reagent Cartridge and lysates to +15 to +25°C before use. If you use the reagents at temperatures outside the recommended range, the kit may not work well.

Protocol

Isolate total RNA according to the protocol below.

❶
- Turn on the instrument.
- Remove Cartridge Rack and Tube Rack (with Elution Tube Rack) from the instrument.
- Click the Run button on the Main Menu Screen to access Sample Ordering Screen 1.
- Follow the software-guided workflow.

❷
- Remove a pre-filled Reagent Cartridge from its bag.
- Equilibrate the Reagent Cartridge to +15 to +25°C (30 min).

⚠ Handle each Reagent Cartridge prior to use as follows:

- Always wear gloves when handling the MagNA Pure Compact cartridge.
- Hold the cartridge only at the barcode imprinted area and the opposite side.
- Avoid touching the sealing foil covering the cartridge wells.
- Avoid touching the two single open wells and do not use them as handles.
- Avoid any foam formation.
- Let the fluid within the cartridge wells settle again completely. If fluid remains under the sealing foil, knock the cartridge bottom gently on a flat lab bench surface. This is especially important for well 3, which contains a small volume of 70 µl Proteinase K.

❸
- Scan the barcode.
- With the two isolated wells pointing away from you, insert all the wells on the Reagent Cartridge into the holes in the Cartridge Rack. Use the guide slots on the rack to help position the cartridge.
- Repeat the steps above for the desired numbers of samples (1 – 8).

❹
- Proceed to Sample Ordering Screen 2.
- Select the appropriate purification protocol from the Protocol menu.
- Select the elution volume (50 µl or 100 µl).

❺
- Insert the appropriate number of Tip Trays (one per purification) into the assigned position in the instrument Tip Rack.

  ⚠ Handle Tip Trays with care to prevent tips or piercing tool from falling out of the tray. Should this happen, discard the respective tip tray and tips. Use the Tip Tray Kit to replace missing Tip Trays.
- Proceed to Sample Ordering Screen 3.

❻ Briefly spin down the DNase (vial 1) and transfer 20 µl of the DNase solution into the bottom of a Sample Tube (one per purification). Insert the open Sample Tubes containing the DNase solution into row 2 (usually the position dedicated for the Internal Control) of the Tube Rack.

❼
- Scan the sample barcode from the primary Sample Tube or enter the sample name.
- Arrange the Sample Tubes containing the sample in the Tube Rack. Make sure that the rim of the tubes are seated firmly in the rack.

❽
- Reinsert the Tube Rack into the instrument.
- Proceed to Sample Ordering Screen 5.

  Sample Ordering Screen 4 only appears if you selected a protocol with internal controls on Sample Ordering Screen 2.

❾
- Scan the barcodes of the Elution Tubes.
- Place the Elution Tubes into the Elution Tube Rack. Make sure that the rim of the tubes are seated firmly in the rack.
- Reinsert the Elution Tube Rack into the instrument.

❿
- Reinsert the Cartridge Rack into the MagNA Pure Compact Instrument.
- Proceed to the Confirmation Screen.

⓫
- On the Confirmation Screen, check the information display. If the information is correct, confirm it by touching the „Confirm Data“ button, close the front cover and start the run.

⓬ • After the purification run has ended, the Result Screen appears, showing the result of the isolation process for each channel:
  • The result will be PASS if the isolation run was completed without any warning or error.
  • The result will be failed if any interruption of the process or error occurred during the run. For each FAIL result, the result screen will show a brief error or warning messages to help you decide whether you may ignore the error or warning. Refer to the troubleshooting section of the MagNA Pure Compact Operator's Manual.

⓭ • Close the Elution Tubes with the supplied Tube Caps and remove the Elution Tube Rack or the capped Elution Tubes immediately after the end of the purification run.
• If not proceeding directly to your downstream application, store RNA samples at –70°C. RNA is stable for at least 6 -12 months, if stored correctly.

⓮ Optionally: Start liquid waste discard. Empty the Waste Tank of the MagNA Pure Compact Instrument after waste handling. Treat liquid waste as potentially infectious (depending on sample material) and hazardous (see Precautions for details).

3. Results

Yield and Purity

RNA was isolated from the sample materials listed in the table below. The elution volume was set to 100 µl. Yield was determined by OD_{260} measurement, purity was determined by $OD_{260/280}$ measurement.

Sample Material	Amount	Average Yield[#]	RNA Purity
Mouse Liver	5 mg	15 – 25 µg	1.9 - 2.1
Mouse Liver	10 mg	30 – 50 µg	1.9 - 2.1
Human Placenta	10 mg	10 – 14 µg	1.9 - 2.1
Cultured Cells (HeLa)	1×10^6	7 – 15 µg	1.9 - 2.1
Whole blood	200 µl	0.7 – 1.0 µg	1.7 – 1.9
WBCs	1×10^6	0.6 - 0.8 µg	1.7 – 1.9

[#]When using an elution volume of 50 µl, the yield will usually be 20 to 30% lower.

For RT-PCR applications, depending on the sensitivity of the assay and the RT-PCR parameters, weak signals may sometimes be observed in late cycles in the RT-minus control PCR (*i.e.* RNA was used as template in a PCR reaction without prior reverse transcription). These are usually too weak to affect the RT-PCR analysis. However, we recommend designing primers based on an exon/exon boundary of the RNA, which should not amplify genomic DNA.

Integrity and Reproducibility

RNA was isolated in parallel from 8 samples of frozen K562 cell pellets containing 1×10^6 cells each. Elution volume was set to 100 µl. Integrity of RNA was shown by analyzing 1 µl of each eluate using a RNA 6000 Nano Chip on the Agilent 2100 Bioanalyzer. All samples revealed 28S and 18S rRNA bands (Fig. 3) with a ratio of 1.9 to 2.2 and an RNA Integrity Number (RIN) of 9.9, indicating intact and highly pure RNA. CV of yield was below 10%.

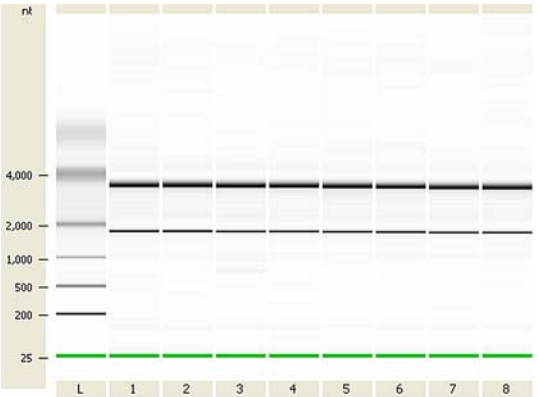

Fig 3: RNA isolated from 1×10^6 K562 cells on the Agilent 2100 Bioanalyzer.

Scalability

Yield: RNA was isolated in replicates of 4 from 2.5, 5, or 10 mg of flash-frozen human placenta. Elution volume was set to 100 µl. Yield was determined by OD_{260} measurement. The resulting values show perfect linear correlation of input sample amount and yield of isolated RNA.

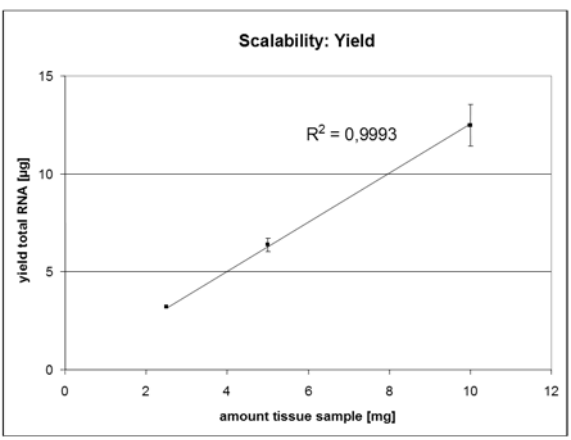

Fig 4: Correlation between input sample amount and RNA yield for human placenta.

- RT-PCR: RNA was isolated in replicates of 4 from 50, 100, or 200 µl of human whole blood samples (containing 6,100 WBCs/µl). Elution volume was set to 100 µl. 5 µl of each eluted RNA sample was used in a LightCycler® Carousel-Based System RT-PCR HybProbe assay, targeting cyclophilin A. The resulting crossing points and the very good linearity confirm the excellent scalability of the isolation procedure. No signs of PCR inhibition were observed.

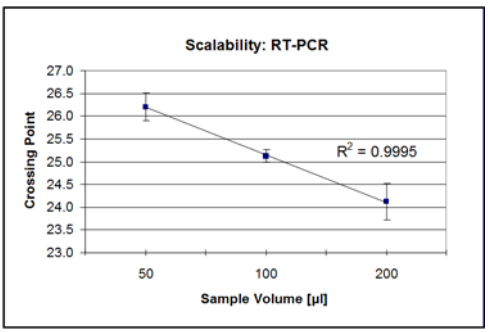

Fig 5: LightCycler® Carousel-Based System HybProbe RT-PCR analysis (targeting cyclophilin A) of RNA samples isolated from different amounts of whole blood.

	Possible Cause	Recommendation
Clogging of beads/problem with magnetic separation of beads	Too much sample material.	Reduce amount of sample material to the values recommended in section "Sample Material".
	MGPs were magnetized prior to use.	Avoid contact between MGPs and magnets. Store kit appropriately.
RNA is degraded	Storage of samples was not optimal.	• Use fresh samples (blood, cells) whenever possible. Do not freeze whole blood before processing. Never collect WBCs from a frozen blood sample. • Use frozen cell pellets only if they have been flash-frozen in liquid nitrogen and stored at -70°C. • Use tissues that have been frozen in liquid nitrogen immediately after removal and stored at –70°C or below. Avoid the use of samples that were stored at ambient temperature. • Use tissue samples that have been properly pre-treated following reagent supplier recommendations (*e.g.*, RNA*later*). • Do not allow cell pellets or tissue samples to thaw before homogenization in Lysis Buffer.
	RNase contamination of Reaction Tips, Sample Tubes, Elution Tubes, or reagents.	Avoid contaminating disposables and reagents with RNases.
Low or no RNA yield	Sample did not contain enough cells; improperly lysed sample.	• Tissue: Weigh the tissue before use. Optimal results are obtained using up to 10 mg tissue. • Cells: Count cultured cells before use. Optimal results are obtained using cell numbers from 5×10^5 to 1×10^6 cells. • Blood: Make sure that the blood is not clotted. Use fresh blood to which anticoagulants were added. • WBCs: Preparation or lysis of WBCs insufficient; work according instructions. See note in section "Sample Material".
	Incorrect storage of samples.	see above
	Buffer temperatures too low.	All buffers have to be at +15 to +25°C; work according instructions. See note in the General Remarks section of "RNA Isolation Protocol".

	Possible Cause	Recommendation
Poor RNA purity	Too much sample material.	Reduce amount of sample material to the values recommended in section “Sample Material”.
	Not enough RNA for correct OD measurement or wrong pH.	Eluates derived from whole blood usually have a low RNA concentration, making a reliable OD measurement difficult. Eluates derived from small amounts of tissue with high RNase content might show the same effect. If the sample shows a high OD at 320 nm, centrifuge sample at max. speed in a microcentrifuge for 1 to 2 min and repeat the OD measurement. Also, the pH of the RNA preparation has an influence on the OD; if necessary spike the eluate with Tris buffer (pH 7 – 8).
High DNA content in eluate	No DNase added.	Make sure to insert the open DNase vial into row 2 of the Tube Rack.
	Too much sample material.	Reduce amount of sample material to the values recommended in section “Sample Material”.
Poor performance in downstream assays (RT-PCR, array)	Poor RNA purity.	Too much sample material used for RNA isolation, adjust input material to the values recommended in section “Sample Material”.
	RT-PCR reagents and protocols were not optimal.	Check RT-PCR reagents and protocols with a positive RNA control.
Eluates show slightly red color	Minimal abrasion from magnetic particles.	Centrifuge at low g-values (approx. 1,000 rpm) to remove fines. The red color does not affect subsequent LightCycler® Carousel-Based System RT-PCR.
Low yields of elution volume	In some cases, only a certain portion of the eluted material is transferred to the elution tubes.	The quality of the isolated nucleic acid is not impacted. We recommend transferring that portion of the eluate still remaining in the reagent cartridge manually to a vial either for storage or a subsequent application, such as PCR.

5. Additional Information on this Product

How this Product Works

MagNA Pure Compact RNA Isolation Kit is used with the MagNA Pure Compact Instrument to purify high-quality, undegraded total RNA from 1-8 samples of mammalian tissue, cultured cells, whole blood or WBCs. The isolated RNA can be eluted into 50 or 100 µl. It meets the quality standards required for highly sensitive and quantitative RT-PCR analysis on the LightCycler® Instruments.

The MagNA Pure Compact System consists of the instrument, reagents and disposables:

- The instrument can perform 1 – 8 isolations of RNA per run.
- The isolation reagents are provided in pre-filled, sealed Reagent Cartridges. Each cartridge contains all reagents required for one isolation, except the DNase (vial 1) for digestion of genomic DNA.
- The reaction tips needed for each isolation are provided in a disposable Tip Tray. In addition, barcoded and sterile tubes for uptake of samples and RNA eluates are supplied.
- For disruption and homogenization of tissue samples or pre-lysis of cultured cells or WBCs outside the MagNA Pure Compact Instrument, prior to RNA isolation, a specific Lysis Buffer (vial 2) is included in the kit.

After instrument setup and starting the software-guided isolation protocol, the MagNA Pure Compact Instrument performs all isolation steps automatically.

Test Principle

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

①	Tissue samples are disrupted and homogenized or cultured cells or WBCs are pre-lysed outside the MagNA Pure Compact Instrument (*e.g.,* using the MagNA Lyser Instrument), applying the special Lysis Buffer containing a chaotropic salt.
②	Sample homogenate or whole blood is lysed by incubation with the Lysis/Binding Buffer containing a chaotropic salt and Proteinase K, which destroys remaining proteins including nucleases.
③	Magnetic Glass Particles (MGPs) are added and nucleic acids are immobilized on the MGPs surfaces.
④	Genomic DNA is degraded by incubation with DNase. This substantially reduces the DNA content of the sample.
⑤	Unbound substances (*e.g.,* protein, cell debris, PCR inhibitors etc.), are removed by several washing steps.
⑥	Purified total RNA is eluted from the MGPs.

Quality Control

- The kit is function tested.
- Model system: Total RNA is isolated from mammalian whole blood, cultured cells and mammalian tissue. Quality of the purified RNA is checked by $OD_{260/280}$ measurement, agarose gel electrophoresis and LightCycler® Carousel-Based System RT-PCR (cyclophilin A as target transcript).
- The kit components are tested for the absence of RNases, according to the current quality control procedures.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
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.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this document, 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 this document the following abbreviations are used:

Abbreviation	Meaning
MGP	magnetic glass particle
OD	optical density
PBS	phosphate buffered saline
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription-PCR
WBC	White Blood Cell

6.2 Changes to Previous Version

- Update of protocol download description
- Editorial changes

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 manuals, please visit and bookmark our homepage, www.roche-applied-science.com and our Special Interest Sites for:

- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System and MagNA Pure LC System): http://www.magnapure.com
- Real-time PCR Systems (LightCycler® 2.0 System, LightCycler® 480 System and Universal ProbeLibrary): http://www.roche-applied-science.com/sis/rtpcr/

	Product	Pack Size	Cat. No.
Instruments and Accessories	MagNA Pure Compact Instrument	1 instrument plus accessories	03 731 146 001
	MagNA Pure Compact Tip Tray Kit	10 tip trays	03 753 166 001
	MagNA Lyser Instrument	1 instrument (220 V) 1 instrument (110 V) (plus Rotor and Rotor Cooling Block)	03 358 976 001 03 358 968 001
	MagNA Lyser Green Beads	100 tubes	03 358 941 001
	LightCycler® 2.0 Instrument	1 instrument plus accessories	03 531 414 001
MagNA Pure Compact Kits for NA Isolation	MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit (32 reactions)	03 730 964 001
	MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume	1 kit (32 reactions)	03 730 972 001
LightCycler® One-Step RT-PCR Kits	LightCycler® RNA Amplification Kit SYBR Green I	1 kit (96 reactions)	12 015 137 001
	LightCycler® RNA Amplification Kit HybProbe	1 kit (96 reactions)	12 015 145 001
	LightCycler® RNA Master SYBR Green I	1 kit (96 reactions)	03 064 760 001
	LightCycler® RNA Master HybProbe	1 kit (96 reactions)	03 018 954 001
cDNA Synthesis Reagents	Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
Associated Kits and Reagents	Red Blood Cell Lysis Buffer	100 ml	11 814 389 001
	Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

6.4 Regulatory Disclaimer

For general laboratory use.

6.5 Trademarks

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SYBR is a registered trademark of Molecular Probes, Inc.

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6.6 License Disclaimer

NOTICE TO PURCHASER: This is a product licensed under patents owned by Qiagen.

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 are committed to providing rapid and effective help.

Please also 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 the research community worldwide.

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

Visit the Roche Applied Science homepage, **www.roche-applied-science.com**, to download or request copies of the following materials:

- Instructions for Use
- Material Safety Data Sheets
- Certificates of Analysis
- Technical Manuals
- Lab FAQS: Protocols and references for life science research

To call, write, fax, or email us, visit the Roche Applied Science homepage and select your home country to display country-specific contact information.

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