For general laboratory use.

MagNA Pure LC RNA Isolation Kit III (Tissue)

Version: 15

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Kit for isolation of total RNA from mammalian tissue using the MagNA Pure LC Instrument

Cat. No. 03 330 591 001 1 kit
up to 192 isolations

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	black	MagNA Pure LC RNA Isolation Kit III (Tissue), Wash Buffer I	To remove PCR inhibitors.	2 bottles, 100 ml each
2	blue	MagNA Pure LC RNA Isolation Kit III (Tissue), Wash Buffer II	To remove residual salts and cellular components.	3 bottles, 100 ml each
3	red	MagNA Pure LC RNA Isolation Kit III (Tissue), Binding Buffer	For RNA binding.	1 bottle, 100 ml
4a	white	MagNA Pure LC RNA Isolation Kit III (Tissue), DNase I	For DNA digestion.	6 glass vials, Lyophilized
4b	white	MagNA Pure LC RNA Isolation Kit III (Tissue), DNase Incubation Buffer	To reconstitute DNase I.	1 bottle, 70 ml
5	black	MagNA Pure LC RNA Isolation Kit III (Tissue), Magnetic Glass Particles	For RNA binding.	6 vials, 8 ml MGP suspension, each
6a	pink	MagNA Pure LC RNA Isolation Kit III (Tissue), Proteinase K	For protein digestion.	6 glass vials, Lyophilized
6b	clear	MagNA Pure LC RNA Isolation Kit III (Tissue), Proteinase K Incubation Buffer	To reconstitute Proteinase K.	1 bottle, 50 ml
7	yellow	MagNA Pure LC RNA Isolation Kit III (Tissue), Elution Buffer	For elution of pure RNA. To reconstitute Paraffin Homogenization Enzyme.	1 bottle, 100 ml
8	green	MagNA Pure LC RNA Isolation Kit III (Tissue), Tissue Lysis Buffer	For tissue lysis and RNA binding.	2 bottles, 70 ml each
9a	pink	MagNA Pure LC RNA Isolation Kit III (Tissue), Paraffin Homogenization Enzyme	To digest cross-linked proteins in formalin-fixed tissues.	1 vial, Lyophilized
9b	white	MagNA Pure LC RNA Isolation Kit III (Tissue), Paraffin Homogenization Buffer	For homogenization of paraffin sections.	1 bottle, 20 ml

Binding Buffer contains a blue ingredient required for clot detection during automated RNA isolation by the MagNA Pure LC Instruments.

Wash Buffer I (Bottle 1) and the Magnetic Glass Particles (MGPs, Bottle 5) have black caps, however the color-coding of the MagNA Pure LC Software and Positioning Frames refer to a caramel cap for the MGPs.

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	black	Wash Buffer I	Store at +2 to +8°C.
2	blue	Wash Buffer II	
3	red	Binding Buffer	
4a	white	DNase I	
4b	white	DNase Incubation Buffer	
5	black	Magnetic Glass Particles	
6a	pink	Proteinase K	
6b	clear	Proteinase K Incubation Buffer	
7	yellow	Elution Buffer	
8	green	Tissue Lysis Buffer	
9a	pink	Paraffin Homogenization Enzyme	
9b	white	Paraffin Homogenization Buffer	

1.3. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Pipettes and nuclease-free, aerosol-resistant tips to predispense samples into the MagNA Pure LC Sample Cartridge
- Centrifuge and suitable nuclease-free reaction tubes
- Vortex mixer to resuspend the MGPs
- Thermal block or water bath
- Homogenization device:
  - MagNA Lyser Instrument* with MagNA Lyser Green Beads*
  - Rotor-stator homogenizer, such as Ultra Turrax or Omni TH 220
  - Mortar/pestle/needle (0.6 mm)

Deparaffinization

- Hemo-De or xylol
- Ethanol, absolute
- SDS, 10%

Stabilization Reagent

- RNA*later*

1.4. Application

For general laboratory use. The MagNA Pure LC RNA Isolation Kit III (Tissue), a General Purpose Reagent (GPR), is designed for use with the MagNA Pure LC 2.0 Instrument to isolate highly purified total RNA from fresh-frozen or formalin-fixed, paraffin-embedded mammalian tissue.

- Unfrozen tissue, stabilized using specific reagents (e.g., RNA*later*), can also be used.
- Purified RNA from fresh tissue can be used for RT-PCR with LightCycler® Instruments* or standard thermal block cyclers, northern blot analysis, and other typical downstream applications in gene-expression analysis.
- The quality of RNA isolated from formalin-fixed, paraffin-embedded tissue sections is suitable for relative quantification of mRNA by RT-PCR, especially using the LightCycler® Instruments. Purified RNA is free of any PCR inhibitors.
- The purified RNA is also an ideal starting material for array experiments.

1.5. Preparation Time

Assay Time

MagNA Pure LC Instrument Setup	approximately 15 minutes
Automated purification of RNA from 32 samples	approximately 84 minutes for fresh-frozen tissue (“RNA Tissue Fresh_frozen” protocol), or approximately 122 minutes for formalin-fixed tissue samples (“RNA Tissue Paraffin” protocol).

No hands-on time is required after set up of the MagNA Pure LC Instruments. Additional hands-on time is required for the manual pre-isolation steps.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

For optimal results in downstream procedures, especially in real-time RT-PCR assays using LightCycler® Instruments, do not process samples larger than this kit is designed to handle.
Optimal amount of sample material:

- 1 to 10 mg of fresh-frozen or unfrozen, stabilized (e.g., treated with RNA*later*) tissue.
- 1 formalin-fixed, paraffin-embedded tissue section (5 to 20 µm) thick.

⚠ ***Do not use more sample material than indicated. This may affect the performance of the isolation process and may lead to clumping and loss of MGPs, or cross-contamination of samples. Due to the high viscosity of certain tissues, using more than 10 mg of tissue may result in blocking of the MagNA Pure LC Reaction Tips.***

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

Control Reactions

Always run appropriate controls with the samples, especially if you want to perform quantification analyses of the eluted RNA samples using real-time PCR/RT-PCR assays with the LightCycler® Instruments.The following control procedures are recommended:

- Positive Control, using a sample material positive for your target.
- Negative Control, using a sample material negative for your target.
- Extraction Control, using PBS or water.
- Internal Control (IC), by adding a defined amount of a control template to all samples to be purified.

ⓘ *The IC is added prior to the purification step and then co-purified and amplified with your target of interest in the same RT-PCR reaction. The IC concept is especially useful for enzyme-based amplification processes such as PCR and RT-PCR, because efficiency of the PCR process may be reduced by inhibitors present in the purified sample material. In addition, the IC is used to compensate for possible losses of your target during purification.*

ⓘ *For quantification assays on the LightCycler® Instruments, use an in vitro-transcribed target molecule with primer-binding sites identical to those of your target sequence, but having a unique probe-binding region that differentiates the IC from the target-specific amplicon. Discriminate the signals derived from your target and the IC by performing a dual-color HybProbe Assay. For detailed information regarding the IC concept, in combination with the LightCycler® Carousel-Based System, read the LightCycler® Technical Note 12/2000 "Absolute Quantification with External Standards and an Internal Control", available at www.lightcycler.com.*

General Considerations

Precautions

- Wash Buffer I (Bottle 1) and Tissue Lysis Buffer (Bottle 8) contain guanidinium salts which are hazardous irritants.

⚠ ***Do not allow them to come in contact with skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If necessary, immediately contact your laboratory supervisor and seek medical assistance. For spilled reagents, dilute the spill with large amounts of water before wiping it up.***

- Do not allow Wash Buffer I (Bottle 1) or the Tissue Lysis Buffer (Bottle 8) to come in contact with sodium hypochlorite (bleach) solution.

⚠ ***This mixture can produce a highly toxic gas.***

- Do not pool reagents from different MagNA Pure LC reagent lots or from different bottles of the same lot.
- Do not use the kit after the expiration date.

Number of Samples

The MagNA Pure LC RNA Isolation Kit III (Tissue) is designed to process up to 192 samples in six batches of 32 from up to 10 mg fresh-frozen or unfrozen, stabilized mammalian tissue, or 96 samples from up to 20 µm sections of formalin-fixed, paraffin-embedded mammalian tissue. When processing fewer than 32 samples at a time, more reagent will be used up and lost in dead volume during each run and the remaining reagents will not be enough to process 192 samples.
Isolation from fresh-frozen and formalin-fixed tissue can be combined, for example:

- 128 total RNA isolations from fresh-frozen tissue and 32 from formalin-fixed tissue sections, or
- 64 total RNA isolations from fresh-frozen tissue and 64 from formalin-fixed tissue sections.

Safety Information

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 varies, the operator must optimize pathogen inactivation by the Tissue Lysis Buffer or take appropriate measures, according to local safety regulations.
- 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 contaminate the reagents with bacteria, virus, or ribonucleases. 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 LabFAQs at lifescience.roche.com.
- Wash hands thoroughly after handling samples and reagents.
- Finish 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. Perform sample preparation, RT-PCR setup, and the RT-PCR run itself in separate locations.

Waste Handling

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

Working Solution

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

Reagent	Preparation	Storage and Stability
DNase I Solution (Vials 4a)	▪ Reconstitute each vial of DNase (Vial 4a) with 10 ml of DNase Incubation Buffer (Bottle 4b). ▪ Close the vial and mix well to completely dissolve the lyophilizate. ▪ One vial of DNase is sufficient for 32 samples.	Store 1 month at +2 to +8°C, or up to 12 months at -15 to -25°C.
Magnetic Glass Particles (Vials 5)	▪ Mix the MGP suspension (Vial 5) thoroughly. ▪ Vortex immediately before use to produce a homogeneous suspension. The beads tend to sediment during storage. ▪ For best results, add the MGPs to the Instrument just before starting the run to minimize sedimentation. ▪ Always use the exact amount of MGPs recommended by the software.	▪ Store at +2 to +8°C. ▪ Do not store the MGP suspension in a Reagent Tub or similar container. ▪ Do not leave the MGP suspension uncovered in the vial or in the Reagent Tub as evaporation may lead to suboptimal purification.
Proteinase K (Vials 6a)	▪ Reconstitute each vial of Proteinase K (Vial 6a) by first adding 3.0 ml Proteinase K Incubation Buffer (Bottle 6b). ▪ Close the vial and mix well to completely dissolve the lyophilizate. ▪ After total solubilization, add an additional 2.0 ml of the buffer to reach the final volume of 5 ml and mix again. *i* *After dissolving the Proteinase K in the Proteinase K Incubation Buffer, the solution may appear turbid. This is caused by stabilizing components added to the Proteinase K. This appearance has no impact on the functionality of the enzyme.* ▪ One vial of Proteinase K is sufficient for 32 fresh-frozen or 16 formalin-fixed tissue samples.	Store 1 month at +2 to +8°C, or up to 12 months at -15 to -25°C.
Paraffin Homogenization Enzyme Solution (Vial 9a)	▪ Reconstitute the Paraffin Homogenization Enzyme lyophilizate (Vial 9a) by adding 4.5 ml of Elution Buffer (Bottle 7). ▪ Mix well to completely dissolve the lyophilizate. ▪ One vial of Paraffin Homogenization Enzyme Solution is sufficient for 96 formalin-fixed, paraffin-embedded sections.	Store the solution in aliquots at -15 to -25°C for up to 1 year. *i* *Place the new label provided in the kit on the vial.*

i *All other solutions are ready-to-use.*

⚠ ***All buffers are clear. Do not use a buffer if it contains a precipitate. If a precipitate is visible, place the bottle at +37°C and mix occasionally until the precipitate is completely dissolved. Stop heating the buffer as soon as the precipitate is completely dissolved. Before using the buffer, equilibrate at +15 to +25°C.***

⚠ ***Equilibrate buffers and working solutions to +15 to +25°C before use. If you use the reagents at temperatures outside the recommended range, the kit may not function properly.***

⚠ ***Use only the reagent amount required for the number of samples.***

⚠ ***Do not store Proteinase K, DNase, or the MGP suspension in a Reagent Tub or similar container. All other reagents remaining in the Reagent Tubs after completion of the run may be used for the next run if performed the same day. Longer storage periods are not recommended.***

2.2. Protocols

Purification Protocol

To perform RNA isolations with the MagNA Pure LC RNA Isolation Kit III (Tissue), two different purification protocols are available. The protocol names listed below should appear on the 'Ordering' sub-tab of the MagNA Pure LC 2.0 Instrument.

Protocol Name	Sample Material	Procedure
RNA Tissue Fresh_frozen	1 to 10 mg of: Fresh-frozen tissue Unfrozen, stabilized tissue	▪ Fresh-frozen tissue samples are lysed and homogenized with a suitable homogenization device. ▪ Transfer of homogenized sample to the MagNA Pure LC Reagent/Sample Stage. ▪ RNA purification is performed automatically by the Instrument.
RNA Tissue Paraffin	5 to 20 µm sections of formalin-fixed, paraffin-embedded tissue	▪ Formalin-fixed, paraffin-embedded tissue sections are homogenized by an overnight incubation with Proteinase K and SDS. ▪ Transfer of homogenized sample to the Reagent/ Sample Stage. ▪ RNA purification is performed automatically by the Instrument.

Both "RNA Tissue" purification protocols enable the eluate to be diluted with 50 to 100 µl Elution Buffer.

⚠ ***When using an elution volume of 50 µl, total RNA yield may be 10 to 20% lower compared to when using 100 µl.***

Pre-Isolation Steps for Fresh-Frozen Tissue

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.
Excessive disruption and homogenization of the tissue lysate will lead to shearing of high-molecular weight genomic DNA and other high molecular weight cellular components, reducing the viscosity of the lysate. Incomplete homogenization will result in significantly reduced RNA yields and may cause clogging of the MagNA Pure LC Reaction Tips.
With some disruption methods, the sample is simultaneously lysed and homogenized, while others require an additional homogenization step.

⚠ ***Always freshly prepare tissue lysates and process them immediately. If necessary, store the lysate at -60°C or below.***

Tissue Disruption Using the MagNA Lyser Instrument

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

1. Add 450 µl Tissue Lysis Buffer (Bottle 8) to a MagNA Lyser Green Beads* tube.

2. Transfer 1 to 10 mg tissue into the tube.
   Start with a minimum of double the amount of tissue and buffer for homogenization since part of the sample lysate (bound between the homogenization beads) cannot be utilized.

3. Set up the MagNA Lyser Instrument as described in the Operator's Manual.

4 Start the disruption cycle, applying speed and time settings appropriate for the specific sample material.

⚠ ***Always optimize the tissue disruption parameters (speed, time) prior to performing the actual RNA purification procedure. Insufficient disruption may lead to poor RNA yields, while excessive disruption may lead to RNA degradation.***

- Refer to the following table for values of exemplary sample materials:

Sample Material	Speed	Time [s]
Liver/Kidney	6,500 rpm	50
Spleen/Tumor Tissue RNA*later*-fixed Tissue		2 × 50[(1)]
Tail/Ear/Skin		2 - 3 × 50[(1)]

5 Incubate samples 30 minutes at +15 to +25°C.

6 Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

7 Transfer 350 µl of the lysate supernatant into the Sample Cartridge.

8 Place the Sample Cartridge on the Reagent/Sample Stage and start the "RNA Tissue Fresh_frozen" protocol, as described in section **RNA Purification Protocol**.

(1) Long disruption cycles may cause degradation of RNA by heat stress. Avoid continuous disruption of cycles of more than 50 seconds. Instead, apply several disruption cycles of 50 seconds maximum. Cool the samples in the MagNA Lyser Rotor Cooling Block supplied with the MagNA Lyser Instrument, or on ice between the disruption cycles.

Tissue Disruption Using a Rotor-Stator Homogenizer

This procedure describes the disruption and homogenization of fresh-frozen, or RNA*later*-fixed tissue using a rotor-stator homogenizer, such as UltraTurrax or Omni TH 220.

1 Lyse and homogenize tissue with 350 µl Tissue Lysis Buffer (Bottle 8) in a rotor-stator homogenizer, following the instrument supplier's instructions. Depending on the type of sample, this takes approximately 5 to 90 seconds.

ⓘ ***Depending on the type of tissue, several disruption cycles may be necessary.***

⚠ ***Always hold and keep the tip of the homogenizer submerged and to one side of the tube to avoid the development of foam.***

2 Incubate the samples 30 minutes at +15 to +25°C.

3 Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

4 Transfer 350 µl of the lysate supernatant into the Sample Cartridge.

5 Place the Sample Cartridge on the Reagent/Sample Stage and start the "RNA Tissue Fresh_frozen" protocol, as described in section **RNA Purification Protocol**.

Tissue Disruption Using Mortar/Pestle/Syringe

This procedure describes disruption and homogenization of fresh-frozen, or RNA*later*-fixed tissue using a mortar, pestle, and syringe.

1 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 suitable for centrifugation.
- Allow the remaining liquid nitrogen to evaporate, but avoid thawing of the tissue sample.

2. Add 350 µl Tissue Lysis Buffer (Bottle 8) to the sample, then homogenize further by passing the sample through a 0.6 mm syringe needle several times.

3. Incubate samples 30 minutes at +15 to +25°C.

4. Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

5. Transfer 350 µl of the lysate supernatant into the Sample Cartridge.

6. Place the Sample Cartridge on the Reagent/Sample Stage and start the "RNA Tissue Fresh_frozen" protocol, as described in section **RNA Purification Protocol**.

Pre-Isolation Steps for Formalin-Fixed, Paraffin-Embedded Tissue Sections

The MagNA Pure LC RNA Isolation Kit III (Tissue) is optimized for the isolation of RNA from paraffin-embedded tissue sections fixed with 10% neutral buffered formalin. Other tissue fixation procedures may affect the RNA yield.

i *Formalin fixation inactivates intracellular RNases. Therefore, paraffin-embedded tissue sections can be handled and stored at +15 to +25°C.*

Deparaffinization on a Microscope Slide

This procedure describes the deparaffinization procedure for one section of formalin-fixed, paraffin-embedded tissue (5 to 20 µm thickness) directly on a microscope slide.

1. Place the slide in a Hemo-De or xylol bath.

2. Incubate for 10 minutes.

3. Tap off excess liquid and place the slide into absolute ethanol for 10 minutes.

4. Change the bath or transfer the slide into fresh absolute ethanol and incubate the slide for an additional 10 minutes.

5. Scratch the deparaffinized section from the slide using a sterile, single-use scalpel and place the section into a 1.5 ml reaction tube.

   i *To avoid tissue dispersing and loss of sample, scratch the section from the microscope slide before it has dried.*

6. Dry the tissue pellet for 10 minutes at +55°C.

7. Add 100 µl Paraffin Homogenization Buffer (Bottle 9b), 16 µl 10% SDS, and 40 µl Paraffin Homogenization Enzyme Solution.

8. Vortex briefly with short intermittent pauses several times.

9. Incubate overnight at +55°C.

10. Centrifuge for 2 minutes at 13,000 × *g* at +15 to +25°C.

11. Transfer 130 µl of the tissue lysate supernatant into the Sample Cartridge.

12 Add 220 µl Tissue Lysis Buffer (Bottle 8) to the sample lysate for a final volume in the Sample Cartridge well of 350 µl.

13 Place the Sample Cartridge on the Reagent/Sample Stage and start the “RNA Tissue Paraffin” protocol, as described in section **RNA Purification Protocol**.

Deparaffinization Within a Reaction Tube

This procedure describes the deparaffinization for one section of formalin-fixed, paraffin-embedded tissue (5 to 20 µm thickness) in a 1.5 ml reaction tube.

1 Add 800 µl Hemo-De or xylol to one 5 to 20 µm tissue section in a 1.5 ml reaction tube.
- Invert several times to mix.

2 Add 400 µl absolute ethanol to the Hemo-De or xylol and mix gently by inverting the tube several times.

3 Centrifuge for 2 minutes at maximum speed (12,000 to 14,000 rpm), then discard the supernatant.

4 Add 1 ml absolute ethanol and mix gently by inverting the tube several times.

5 Centrifuge for 2 minutes at maximum speed and discard the supernatant.

6 Blot the tube briefly on paper towel to remove residual ethanol.

7 Dry the tissue pellet for 10 minutes at +55°C.

8 Add 100 µl Paraffin Homogenization Buffer (Bottle 9b), 16 µl 10% SDS, and 40 µl Paraffin Homogenization Enzyme Solution.

9 Vortex briefly with short intermittent pauses several times.

10 Incubate overnight at +55°C.

11 Centrifuge for 2 minutes at 13,000 × *g* at +15 to +25°C.

12 Transfer 130 µl of the tissue lysate supernatant into the Sample Cartridge.

13 Add 220 µl Tissue Lysis Buffer (Bottle 8) to the sample lysate for a final volume in the Sample Cartridge well of 350 µl.

14 Place the Sample Cartridge on the Reagent/Sample Stage and start the “RNA Tissue Paraffin” protocol, as described in section **RNA Purification Protocol**.

RNA Isolation Protocol

This procedure is designed to process 32 samples at the same time. If you are processing fewer samples, the software will reduce the volumes of all solutions accordingly, see **'Stage Setup' sub-tab of the MagNA Pure LC 2.0 Instrument**. The software automatically calculates the necessary amounts of reagents and disposable plastics and guides you through the setup. The Disposable Lockbar, for securing the Sample Cartridge, Reagent Tubs, and Reaction Tips, must be closed to start the Instrument.

Isolate RNA according to the following protocol:

Start Instrument and Software
▪ Turn on the Instrument; the MagNA Pure LC 2.0 Software starts automatically. ▪ Log in and then navigate to the 'Ordering' sub-tab.

Select the appropriate protocol:

If you are starting with.....	**Then use the....**
Fresh-frozen or unfrozen, stabilized tissue samples	"RNA Tissue Fresh_frozen" protocol
Formalin-fixed, paraffin-embedded tissue sections	"RNA Tissue Paraffin" protocol
Follow the instructions of the Software and specify the name and number of samples. Type in the Sample Volume and Elution Volume. The software will calculate how much of each reagent is required.	
Fill the Reagent Tubs	
Before starting the isolation procedure, fill all Reagent Tubs outside the Instrument with the required amount of reagents equilibrated to +15 to +25°C.	
Fill each Reagent Tub with the volume listed on the 'Stage Setup' sub-tab.	
▪ Close Reagent Tubs with the Tub Lids to prevent evaporation of the reagents. *Reagent Tubs are not suitable for long-term storage of reagents.*	
▪ Load the exact amount of MGPs (as listed on the 'Stage Setup' sub-tab) on to the Instrument just before the run starts. This will prevent them from sedimenting.	
Set Up Reagent Tubs and Disposables on the Reagent/Sample Stage	
Use the information of the 'Stage Setup' sub-tab to place all disposable plastics and reagents within Reagent Tubs, necessary for the batch run on the Reagent/Sample Stage.	
A colored "Positioning Frame"* that can be placed on the Reagent Reservoir Rack to aid correct loading of the reagents is available with the MagNA Pure LC Disposables Starter Set.	
Load the Samples	
▪ Transfer the Sample Cartridge containing the samples or lysates to the MagNA Pure LC Instrument. ▪ Close the Disposable Lockbar.	
Start the Batch Run	
▪ On the 'Stage Setup' sub-tab, confirm the correct placement of all disposable plastics and reagents, by selecting the respective button on the Reagent/Sample Stage Area. ▪ Select the 'Start' button to start the automated RNA isolation procedure. The Instrument will automatically dispense all reagents and process the samples.	

Storage of RNA Eluates

- Close the Storage Cartridge with a MagNA Pure LC Cartridge Seal* and store the RNA at -60°C or below (stable for at least several weeks).
- For long-term storage, it is recommended to store the RNA in aliquots in screw-capped tubes at -60°C or below.
  ⚠ ***Avoid repeated freezing and thawing.***
- To ensure greatest possible stability of the eluted RNA, immediately proceed with RT-PCR setup.
- Do not store the eluted RNA in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.
- After thawing eluates, mix gently by pipetting up and down ten times before performing any downstream steps, such as RT-PCR, or OD measurements. If nucleic acids are not premixed and distributed homogeneously in solution, results may not be reproducible in subsequent assays.

Post Elution Steps

The MagNA Pure LC Instruments can set up RT-PCR, reverse transcription, and PCR reactions by pipetting RNA samples and master mixes for RT-PCR, RT, or PCR into either LightCycler® Capillaries* or standard PCR tubes or plates.
ⓘ *See the MagNA Pure LC Operator's Manuals for recommended plates.*

For Post Elution procedures, you can place LightCycler® Capillaries into the removable MagNA Pure LC Cooling Block, LC Centrifuge Adapters*, or the MagNA Pure LC Cooling Block, LC Sample Carousel*. Alternatively, you can place a LightCycler® 480 Multiwell Plate 96* into the MagNA Pure LC Cooling Block, 96-well PCR Plate*, in combination with the MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter*. You can program the Post Elution steps either before you perform the isolation procedure, or after it is finished.
ⓘ *See the MagNA Pure LC Operator's Manuals, for details on how to set up a Post Elution run.*

3. Results

Integrity

Total RNA was isolated from either 10 mg fresh-frozen or RNA*later*-treated mouse liver using the “RNA Tissue Fresh_frozen” purification protocol. RNA integrity was assessed using denaturing agarose gels stained with ethidium bromide. All RNA samples showed good integrity, as indicated by intact 18S and 28S ribosomal RNA bands (Fig. 1).

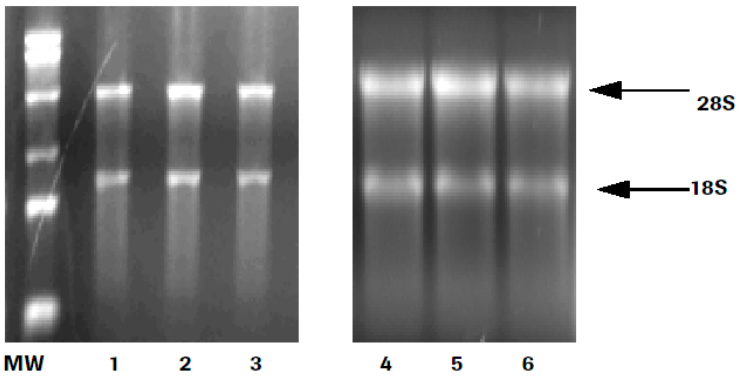

Fig. 1: Denaturing agarose gel electrophoresis of RNA isolated from fresh-frozen (lanes 1 to 3) or RNAlater-treated (lanes 4 to 6) mouse liver.

Yield

RNA was prepared from either fresh-frozen and RNA*later*-treated tissue or paraffin-embedded tissue sections. Yields were determined by OD_{260} measurement and agarose gel electrophoresis, see **Typical Results Table**.

Purity

RNA was purified from different amounts and types of tissue sample materials. In typical experiments, RNA had an $OD_{260/280}$ ratio of 2.0 ± 0.2, indicating highly purified RNA, see **Typical Results Table**.
In all experiments, a “minus-RT” control PCR targeting cyclophilin A was performed for all of the samples. After 40 cycles, no signal was observed in RT-PCR analysis using the LightCycler® Carousel-Based System, indicating the total absence of contaminating genomic DNA (Fig. 2).

Depending on the sensitivity of the RT-PCR and on the RT-PCR parameters, occasionally weak signals rising in the later cycles may be observed in the minus-RT control PCR. 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.

Typical Results From Different Sample Materials			
Tissue Type	Amount [mg]	RNA Yield [µg]	Purity [$OD_{260/280}$]
Fresh-frozen tissue	10	5 - 50	2.0 ± 0.2
RNA*later*-treated tissue		5 - 50	
Formalin-fixed, paraffin embedded tissue	5 µm	1 - 3	

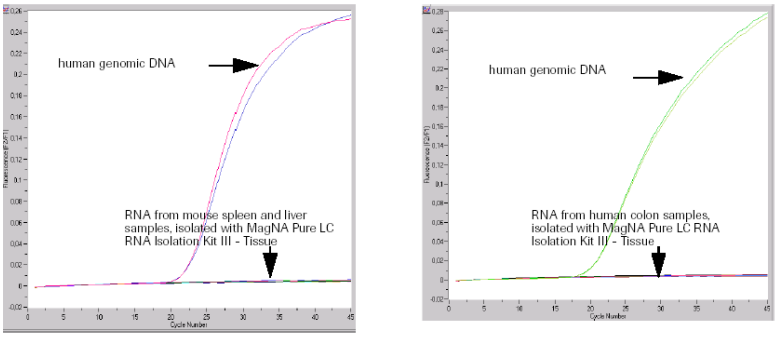

Fig. 2: PCR analysis using the LightCycler® Carousel-Based System (HybProbe format) of total RNA isolated with the MagNA Pure LC RNA Isolation Kit III - Tissue targeting cyclophilin A revealed no detectable residual genomic DNA. As a control, 10 ng of human genomic DNA were amplified in parallel.

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads or presence of beads in Storage Cartridge.	Too much sample material or inefficient homogenization method.	Reduce amount of sample material to the values indicated in the section **Sample Material**.
		Use recommended homogenization methods, see section **Pre-Isolation Steps for Fresh-Frozen Tissue**.
	MGPs were magnetized prior to use.	Avoid contact between MGPs and magnets prior to use.
		Store kit properly.
RNA is degraded.	Samples stored incorrectly.	Use fresh tissue, or tissues that have been frozen in liquid nitrogen immediately after harvesting and stored at -60°C or below.
		Avoid the use of samples that have been stored extensively at +15 to +25°C.
		Use tissues that have been pretreated according to the reagent supplier's recommendations, such as RNA*later*.
		Do not allow the samples to thaw before homogenization in Tissue Lysis Buffer.
	Homogenization too intensive.	Shorten homogenization.
	RNase contamination of Reaction Tips, Reagent Tubs, Sample Cartridges, or reagents.	Avoid contaminating disposables and reagents with RNases.
Poor or no RNA yield.	Sample did not contain enough cells; improperly lysed sample.	Weigh tissue before use. *Optimal results are obtained using up to 10 mg tissue.*
	Samples stored incorrectly.	Use fresh tissue, or tissues that have been frozen in liquid nitrogen immediately after harvesting and stored at -60°C or below.
		Avoid the use of samples that have been stored extensively at +15 to +25°C.
		Use tissues that have been pretreated according to the reagent supplier's recommendations, such as RNA*later*.
		Do not allow the samples to thaw before homogenization in Tissue Lysis Buffer.
	Reagents were placed incorrectly on the Reagent/ Sample Stage.	Ensure that all reagents are in the correct positions on the Reagent/Sample Stage.
Poor RNA purity.	Too much sample material.	Reduce amount of sample material to the values recommended in the section **Sample Material**.
Poor RT-PCR performance.	Poor RNA purity.	Use less sample material for the RNA isolation. Reduce amount of sample material to the values recommended in the section **Sample Material**.
	RT-PCR reagents and protocol were not optimal.	Check RT-PCR reagents and protocols with a positive RNA control.
Poor RT-PCR performance with RNA isolated from formalin-fixed, paraffin-embedded sections.	Amplicon too large.	Do not use amplicons >200 bp, considering the degradation of the nucleic acids in formalin-fixed tissues.

5. Additional Information on this Product

5.1. Test Principle

The tissue samples or paraffin sections are first homogenized using the MagNA Lyser Instrument or other suitable homogenization device, using the Tissue Lysis Buffer supplied in the kit. Homogenization of formalin-fixed, paraffin-embedded tissue sections is performed by an overnight incubation with Proteinase K. Sample lysates are then transferred to the MagNA Pure LC Instrument. A Proteinase K step enhances the cell lysis and digests proteins. RNA then binds to the surface of the added Magnetic Glass Particles (MGPs) under chaotropic salt conditions. Unbound substances are removed by several washing steps, and purified RNA is eluted using a low-salt buffer (Fig. 3).

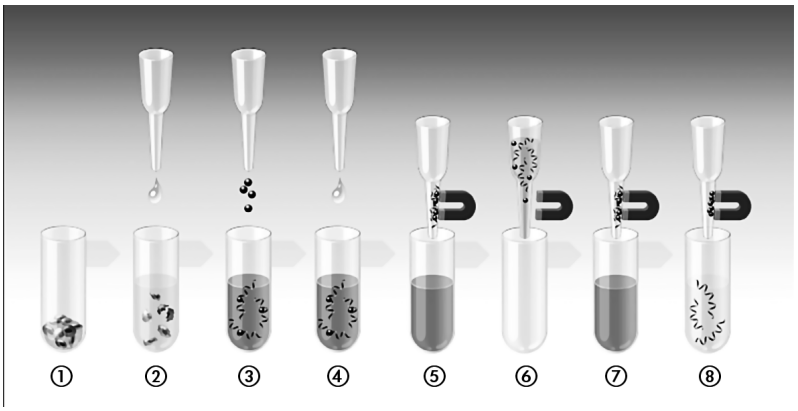

Fig. 3: Principle steps of a MagNA Pure LC RNA isolation.

The principle steps of a MagNA Pure LC RNA isolation procedure are:

① Sample lysate is placed into the wells of the Sample Cartridge.

② Binding Buffer and Proteinase K are added to the sample lysate, resulting in full cell lysis, digestion of proteins, and release of RNA.
– RNases are denatured.

③ RNA binds to the silica surface of the added MGPs due to the chaotropic salt conditions, isopropanol, and the high ionic strength of the Binding Buffer.

④ Genomic DNA is removed by incubation with DNase I.

⑤ MGPs with bound RNA are magnetically separated from the residual lysed sample.

⑥ MGPs with bound RNA are washed repeatedly with Wash Buffer to remove unbound substances, such as proteins (nucleases), cell membranes, and PCR inhibitors, such as heparin or hemoglobin, and to reduce the chaotropic salt concentration.

⑦ MGPs with bound RNA are magnetically separated from the Wash Buffer containing residual sample debris.

⑧ Purified RNA is eluted from the MGPs in the wells of the Elution Cartridge.
– MGPs are retained in the Reaction Tip and discarded.

Automated RNA Isolation Using the MagNA Pure LC Instruments

RNA Tissue Fresh_frozen Protocol

The basic steps of the protocol are:

① Dispense all required reagents into the Processing Cartridge.

② Dispense Elution Buffer into the Elution Cartridge (Heating Unit).

③ Add Binding Buffer and Proteinase K to the lysate and mix.

④ Transfer lysate mix into the MGP suspensions, mix, incubate, and separate particles.

⑤ Transfer MGPs into DNase solution, incubate, and separate particles.

⑥ Transfer MGPs into Wash Buffer I, mix, and separate particles.

⑦ Transfer MGPs into Wash Buffer II, mix, and separate particles.

⑧ Repeat Step 7.

⑨ Repeat Step 7.

⑩ Transfer MGPs into Elution Buffer, mix, incubate, and elute RNA.
– Discard MGPs.

⑪ Transfer eluate to the Storage Cartridge (Cooling Unit I).

RNA Tissue Paraffin Protocol

The basic steps of the protocol are:

① Dispense all required reagents into the Processing Cartridge.

② Dispense Elution Buffer into the Elution Cartridge (Heating Unit).

③ Add Binding Buffer to the lysate and mix.

④ Transfer lysate mix into the MGP suspensions, mix, incubate, and separate particles.

⑤ Transfer MGPs into DNase solution, incubate, and separate particles.

⑥ Transfer MGPs into Wash Buffer I, mix, and separate particles.

⑦ Transfer MGPs into Wash Buffer II, mix, and separate particles.

⑧ Transfer MGPs into Proteinase K solution, mix, and separate particles.

⑨ Transfer MGPs into Wash Buffer I, mix, and separate particles.

⑩ Transfer MGPs into Wash Buffer II, mix, and separate particles.

⑪ Repeat Step 10.

⑫ Transfer MGPs into Elution buffer, mix, incubate, and elute RNA.
– Discard MGPs.

⑬ Transfer eluate to the Storage Cartridge (Cooling Unit I).

5.2. Quality Control

- RNA is isolated from frozen mouse liver and formalin-fixed paraffin sections, using the appropriate purification protocol. Quality of the purified RNA is checked using $OD_{260/280}$ readings, and RT-PCR/PCR analysis on the LightCycler® System.
- The kit components are tested for the absence of RNases.

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

- Chapter **Quality Control** has been updated.
- 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.
Accessories general (hardware)		
MagNA Pure LC Cooling Block, LC Centrifuge Adapters	1 cooling block	12 190 664 001
MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter	1 adapter	05 323 983 001
MagNA Pure LC Cooling Block, LC Sample Carousel	1 cooling block	12 189 704 001
MagNA Pure LC Cooling Block, 96-well PCR Plate	1 cooling block	12 189 674 001
Consumables		
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
MagNA Pure LC Disposable Starter Set	1 set	03 005 488 001
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
MagNA Lyser Instrument	1 instrument, 120 V	03 358 968 001
	1 instrument, 230 V, *Not available in US*	03 358 976 001
Reagents, kits		
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001

6.4. Trademarks

MAGNA LYSER, MAGNA PURE and LIGHTCYCLER are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

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

Visit **lifescience.roche.com**, to download or request copies of the following Materials:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

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