For general laboratory use.

MagNA Pure RNA Tissue Lysis Buffer

Version: 05
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Cat. No. 03 604 721 001	1 bottle 70 ml

Store the buffer at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	MagNA Pure RNA Tissue Lysis Buffer	▪ For tissue lysis and homogenization. ▪ For use with the MagNA Pure 96 Cellular RNA Large Volume Kit*	1 bottle, 70 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the buffer is stable through the expiration date printed on the label.
The buffer is shipped at +15 to +25°C.

Vial / Bottle	Cap	Label	Storage
1	green	MagNA Pure RNA Tissue Lysis Buffer	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Pipettes and nuclease-free, aerosol-resistant tips
- Centrifuge and suitable nuclease-free reaction tubes
- Vortex mixer
- 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)

The MagNA Pure RNA Tissue Lysis Buffer is additional buffer to be used with the MagNA Pure 96 Cellular RNA Large Volume Kit.

1.4. Application

The MagNA Pure RNA Tissue Lysis Buffer is designed for the following applications:

- Lysis and homogenization of fresh-frozen tissue samples using a tissue homogenization device, such as the MagNA Lyser Instrument*, a rotor-stator homogenizer, or mortar/pestle/syringe. Tissue Lysis Buffer completely lyses the cells, resulting in release of RNA. RNases are effectively inhibited.
- Provides stabilization of total RNA within sample lysates.
- Compatible with total RNA purification with the MagNA Pure 96 Instrument.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

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

⚠ ***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 tissue than recommended may result in blocking of the MagNA Pure Tips.***

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

Control Reactions

⚠ ***Always run appropriate controls.***

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

- 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.

ⓘ *For additional information, see the Instructions for Use of the MagNA Pure 96 Cellular RNA Large Volume Kit.*

General Considerations

Precautions

- Tissue Lysis Buffer contains guanidinium salts and DTT which are hazardous irritants.

  ⚠ ***Do not let the Tissue Lysis Buffer 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 the Tissue Lysis Buffer to come in contact with sodium hypochlorite (bleach) solution.

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

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.
- 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.

2.2. Protocols

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 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.***

See the Instructions for Use of the MagNA Pure 96 Cellular RNA Large Volume Kit for the detailed pre-isolation steps, the RNA isolation procedure, and kit-specific troubleshooting hints.

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. Transfer up 10 mg (or up to 25 mg) tissue sample into a MagNA Lyser Green Beads tube containing 400 µl (or 800 µl) Tissue Lysis Buffer.

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

3. 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)]

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

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

6. Proceed with the protocol of the MagNA Pure 96 Cellular RNA Large Volume Kit.

(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 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. Proceed with the protocol of the MagNA Pure 96 Cellular RNA Large Volume Kit.

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 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. Proceed with the protocol of the MagNA Pure 96 Cellular RNA Large Volume Kit.

3. Troubleshooting

Refer to the Instructions for Use of the MagNA Pure 96 Cellular RNA Large Volume Kit for details on how to troubleshoot experimental outcomes using the Tissue Lysis buffer for purifying total RNA with the MagNA Pure 96 Instrument.

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

New product name.
Update ordering information.
MagNA Pure 96 Instrument specific information added.
Editorial changes.

4.3. 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.
Reagents, kits		
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001
MagNA Pure 96 Cellular RNA Large Volume Kit	1 kit, 3 sets 3 x 96 isolations	05 467 535 001

4.4. Trademarks

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

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

For general laboratory use.

4.7. Safety Data Sheet

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

4.8. 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 **documentation.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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