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

High Pure RNA Isolation Kit

Version: 15
Content Version: November 2023

For small-scale (mini) preparations of RNA.

Cat. No. 11 828 665 001 1 kit
50 isolations

Store the kit at +15 to +25°C.

1. General Information ... 3
1.1. Contents ... 3
1.2. Storage and Stability ... 4
Storage Conditions (Product) ... 4
1.3. Additional Equipment and Reagent required ... 4
1.4. Application ... 4
1.5. Preparation Time ... 4
Assay Time ... 4
2. How to Use this Product ... 5
2.1. Before you Begin ... 5
Sample Materials ... 5
General Considerations ... 5
Handling requirements and precautions ... 5
Safety Information ... 5
Laboratory procedures ... 5
Waste handling ... 5
Working Solution ... 6
2.2. Protocols ... 6
Isolation of total RNA from 1×10^6 cultured cells ... 6
Isolation of total RNA from 200 to 500 µL human whole blood ... 7
Isolation of total RNA from 1×10^8 yeast cells ... 7
Isolation of total RNA from 1×10^9 bacterial cells (gram positive and gram negative) ... 8
3. Results ... 8
Sensitivity ... 8
4. Troubleshooting ... 9
5. Additional Information on this Product ... 10
5.1. Test Principle ... 10
How this product works ... 10
5.2. Quality Control ... 10
6. Supplementary Information ... 11
6.1. Conventions ... 11
6.2. Changes to previous version ... 11
6.3. Ordering Information ... 11
6.4. Trademarks ... 12
6.5. License Disclaimer ... 12
6.6. Regulatory Disclaimer ... 12
6.7. Safety Data Sheet ... 12
6.8. Contact and Support ... 12

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Content
1	green	High Pure RNA Isolation Kit, Lysis/Binding Buffer	Contains 4.5 M guanidine-HCl, 50 mM Tris-HCl, 25% polidocanol (w/v).	1 bottle, 25 mL
2	–	High Pure RNA Isolation Kit, DNase I, recombinant	Lyophilized	1 vial, 10 KU
3	white	High Pure RNA Isolation Kit, DNase Incubation Buffer	Contains 1 M NaCl, 20 mM Tris-HCl, and 10 mM $MnCl_2$, pH 7.0 (+25°C).	1 bottle, 10 mL
4	black	High Pure RNA Isolation Kit, Wash Buffer I	Contains 5 M guanidine-HCl and 20 mM Tris-HCl, pH 6.6 (+25°C); final concentrations after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 33 mL
5	blue	High Pure RNA Isolation Kit, Wash Buffer II	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C); final concentrations after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 10 mL
6	colorless	High Pure RNA Isolation Kit, Elution Buffer	Water, PCR Grade	1 bottle, 30 mL
7	–	High Pure RNA Isolation Kit, High Pure Filter Tubes	For use of up to 700 µL sample volume.	1 bag, 50 polypropylene filter tubes with two layers of glass fiber fleece
8	–	High Pure RNA Isolation Kit, Collection Tubes	For RNA isolation.	1 bag, 50 polypropylene tubes, 2 mL each

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a +37°C water bath until the precipitates have dissolved.***

The buffers can show a slight yellow color. This will have no impact on the function of the buffer.

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	green	Lysis/Binding Buffer	Store at +15 to +25°C. ⚠ ***Storage at +2 to +8°C or −15 to −25°C will adversely impact nucleic acid isolation due to the formation of precipitates in the solutions and may result in reduced binding efficiency.***
2	–	DNase I, recombinant	
3	white	DNase Incubation Buffer	
4	black	Wash Buffer I	
5	blue	Wash Buffer II	
6	colorless	Elution Buffer	
7	–	High Pure Filter Tubes	Store at +15 to +25°C.
8	–	Collection Tubes	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Sterile microcentrifuge tubes, 1.5 mL

For the isolation of total RNA from human blood

- Red Blood Cell Lysis Buffer*
- PBS*

For the isolation of total RNA from yeast

- Lyticase (0.5 mg/mL)
- PBS*

For the isolation of total RNA from bacteria

- Lysozyme* (stock solution 50 mg/mL, store aliquots at −15 to −25°C)
- 10 mM Tris, pH 8.0

1.4. Application

The High Pure RNA Isolation Kit is designed for the purification of total RNA from cultured cells. Other sample materials, such as blood, yeast, and bacteria require an additional specific pre-lysis treatment, which is described in the protocol section.

- Due to the integrated DNase digestion step, contamination of the isolated RNA with residual genomic DNA is mostly avoided.
- In addition, RNA is suited for other techniques, such as northern blotting, RNase protection, and primer extension.
- Up to 24 samples can be processed simultaneously in approximately 1 hour. Thus, the isolation procedure is less time consuming compared with alternative methods which require extraction with organic solutions, RNA precipitation, or ultracentrifugation.

1.5. Preparation Time

Assay Time

Total time	Approximately 1 hour (24 samples simultaneously).

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolation of RNA from a variety of sample materials:
- 10^6 cultured cells
- 200 to 500 µL human blood
- 10^8 yeast cells
- 10^9 bacterial cells (gram positive or gram negative)

General Considerations

Handling requirements and precautions

⚠ ***Binding Buffer and Wash Buffer I contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
- Never store or use the Binding Buffer and Wash Buffer I near human or animal food.
- Avoid contact of the Binding Buffer and Wash Buffer I with the skin, eyes, or mucous membranes. If contact does occur, immediately wash the affected area with a large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.
- Do not use any modified ethanol.
- Do not pool reagents from different lots or from different bottles of the same lot.
- Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening, store all bottles in an upright position.
- Do not allow the Binding Buffer and Wash Buffer I to mix with sodium hypochlorite found in commercial bleach solutions. 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 Lysis/Binding 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.
- Use only calibrated pipettes.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipettes and nuclease-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 PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR setup. Sample preparation, PCR/RT-PCR setup, and the PCR/RT-PCR run itself should also be performed 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 on documentation.roche.com, or upon request from the local Roche office.

Working Solution

Prepare the following working solutions:

Content	Reconstitution / preparation	Storage and stability	For use in...
DNase I (Vial 2)	Dissolve DNase I in 0.55 mL Elution Buffer.	▪ Store in aliquots at −15 to −25°C. ▪ Stable for 12 months.	Removal of contaminating DNA.
Wash Buffer I (Bottle 4)	Add 20 mL absolute ethanol to Wash Buffer I and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until the expiry date printed on kit label.	Removal of residual impurities.
Wash Buffer II (Bottle 5)	Add 40 mL absolute ethanol to Wash Buffer II and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until the expiry date printed on kit label.	Removal of residual impurities.

2.2. Protocols

Isolation of total RNA from 1 × 10^6 cultured cells

ℹ *See Section* **Working Solution** *for additional information on preparing solutions.*

1. Resuspend cells in 200 µL PBS.

2. Add 400 µL Lysis/Binding Buffer and vortex for 15 seconds.

3. Insert one High Pure Filter Tube into one Collection Tube.
   – Pipette the entire sample into the upper reservoir of the Filter Tube (maximum 700 µL).

4. Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   – Centrifuge 15 seconds at 8,000 × *g*.

5. After centrifugation:
   – Remove the Filter Tube from the Collection Tube; discard the flow through liquid, and again insert the Filter Tube into the used Collection Tube.

6. After re-inserting the Filter Tube:
   – For each sample, pipette 90 µL DNase Incubation Buffer into a sterile reaction tube; add 10 µL DNase I, mix, and pipette the solution onto the glass filter fleece in the upper reservoir of the filter tube.
   – Incubate for 15 minutes at +15 to +25°C.

7. Add 500 µL Wash Buffer I to the upper reservoir of the Filter Tube assembly and centrifuge 15 seconds at 8,000 × *g*.
   – Discard the flow through and insert the Filter Tube into the used Collection Tube.

8. Add 500 µL Wash Buffer II to the upper reservoir of the Filter Tube assembly and centrifuge 15 seconds at 8,000 × *g*.
   – Discard the flow through and insert the Filter Tube into the used Collection Tube.

9 Add 200 µL Wash Buffer II to the upper reservoir of the Filter Tube assembly and centrifuge for 2 minutes at maximum speed (approximately 13,000 × *g*) to remove any residual Wash Buffer.

The extra centrifugation time ensures removal of residual Wash Buffer.

10 Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 mL microcentrifuge tube.

11 To elute the RNA:
- Add 50 to 100 µL Elution Buffer to the upper reservoir of the Filter Tube.
- Centrifuge the tube assembly for 1 minute at 8,000 × *g*.

12 The microcentrifuge tube now contains the eluted RNA.
- Use the eluted RNA directly in RT-PCR or store the eluted RNA at −80°C for later analysis.

Isolation of total RNA from 200 to 500 µL human whole blood

Due to the modest content of RNA in leukocytes, use the isolated RNA exclusively in RT-PCR. Use EDTA-preserved fresh whole blood. Erythrocytes are lysed by hypotonic lysis. We recommend the application of the Red Blood Cell Lysis Buffer as described in the following steps.

1 Add 1 mL Red Blood Cell Lysis Buffer to a sterile 1.5 mL microcentrifuge tube.

2 Add 500 µL human whole blood and mix by inversion.

Do not vortex.

3 Place the tube on a rocking platform or gyratory shaker for 10 minutes at +15 to +25°C.

Alternatively, manually invert the sample periodically for 10 minutes.

4 Centrifuge for 5 minutes at 500 × *g* in a standard tabletop centrifuge.

5 With a pipette, carefully remove and properly dispose of the clear, red supernatant.
- Add 1 mL Red Blood Cell Lysis Buffer to the white pellet and mix by flicking the tube until the pellet is resuspended.

Do not vortex.

6 Centrifuge for 3 minutes at 500 × *g*.
- Carefully remove and properly dispose of the supernatant, particularly the red ring of blood cell debris that forms around the outer surface of the white pellet.

7 Resuspend the white pellet in 200 µL PBS and follow the protocol **Isolation of total RNA from cultured cells**, starting with Step 2.

Isolation of total RNA from 1 × 10^8 yeast cells

Harvest cells during the mid-log or late-log phase of growth (OD_{600} ≤2.0). The cell number can be counted in a hemocytometer chamber or determined by measuring the optical density at 600 nm in a spectral photometer. Use a dilution which gives a A_{600} of 0.1 to 0.15/mL (0.1 A_{600} corresponds to approximately 2 × 10^6 cells).

1 Collect the sample by centrifugation at 2,000 × *g* for 5 minutes in a standard tabletop centrifuge.

2 Add 10 µL Lyticase (0.5 mg/mL); incubate for 15 minutes at +30°C.

3 Follow the protocol **Isolation of total RNA from cultured cells**, starting with Step 2.

Isolation of total RNA from 1 × 10^9 bacterial cells (gram positive and gram negative)

1. Collect the sample by centrifugation at 2,000 × *g* for 5 minutes in a standard tabletop centrifuge.
   – Resuspend the pellet in 200 µL 10 mM Tris, pH 8.0.
2. Add 4 µL Lysozyme (50 mg/ml); incubate for 10 minutes at +37°C.
3. Add 400 µL Lysis/Binding Buffer and mix well.
4. Insert the High Pure Filter Tube into the Collection Tube.
   – Pipette the sample into the upper reservoir of the Filter Tube.
5. Centrifuge for 15 seconds at 8,000 × *g*; discard the flow through and again insert the Filter Tube into the used Collection Tube.
6. Pipette 90 µL DNase Incubation Buffer into a sterile microcentrifuge tube, add 10 µL DNase I, mix, and pipette the solution into the upper reservoir of the Filter Tube.
   – Incubate for 60 minutes at +15 to +25°C.
7. Follow the protocol **Isolation of total RNA from cultured cells**, starting with Step 7.

3. Results

Isolated total RNA can be used directly in first-strand cDNA synthesis. Depending on the expression level of the target mRNA to be analyzed, use 1 to 10 µL in the RT reaction.

Sensitivity

Fresh human blood was collected from two donors with WBC = 10.7 × 10^3 cells/µL and WBC = 4.8 × 10^3 cells/µL. Each isolation was performed with 12 replicates followed by a duplicated analysis on the LightCycler® 480 Instrument II using the LightMix® Kit human glucose-6-phosphate dehydrogenase (G6PDH). Isolation was performed according to the Instructions for Use of the respective kit followed by quantitative analysis of G6PDH on the LightCycler® 480 Instrument II.

Sample	WBC count	Mean Cp	SD	Input volume	Eluate volume	Instrument
Fresh human whole blood	4.8 × 10^3 cells/µL	25.08	0.51	200 µL	5 µL	LightCycler® 480 Instrument II
	10.7 × 10^3 cells/µL	23.48	0.39			

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity.	Kit stored under suboptimal conditions.	Store kit at +15 to +25°C at all times.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and to avoid contamination.
	Ethanol not added to Wash Buffers.	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always label Wash Buffer I and Wash Buffer II bottles to indicate whether ethanol has been added.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Poor elution of nucleic acids with water.	Water has the wrong pH.	If you use your own water or buffer to elute nucleic acids from the Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbancey ($A_{260\ nm}$) reading of product too high.	Glass fibers, which can coelute with nucleic acid, scatter light.	▪ Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 minute at maximum speed. ▪ Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Low RNA yield.	High levels of RNase activity.	Be careful to create an RNase-free working environment.
		Process starting material immediately or store it at −80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at −80°C.

5. Additional Information on this Product

5.1. Test Principle

How this product works

Isolation of RNA is a prerequisite for the analysis of gene expression. Frequently, applied techniques, such as reverse transcriptase-PCR (RT-PCR), northern blotting, RNase protection, and primer extension require the use of intact, undegraded RNA from different sample materials, such as cultured cells, blood, yeast, and bacteria.

Samples are lysed and homogenized in the presence of chaotropic salts, then applied to the spin filter tube. Nucleic acids bind specifically to the surface of the filter. Co-purified DNA is ultimately digested with DNase I. The bound RNA is purified from salts, proteins, digested DNA, and other impurities by washing steps, followed by an elution.

① Cultured cells are lysed by a special Lysis/Binding Buffer. At the same time, RNases are inactivated.

Other sample materials require a specific pre-lysis treatment.

② Nucleic acids are bound to the glass fibers pre-packed in the High Pure Filter Tube.

③ Residual contaminating DNA is digested by DNase I and applied directly onto the glass fiber fleece.

④ Bound nucleic acids are washed with a special Wash Buffer to get rid of RT-PCR inhibitory contaminants.

⑤ Further washing of bound nucleic acids purifies them from salts, proteins, and other cellular impurities.

⑥ RNA is recovered using the Elution Buffer.

5.2. Quality Control

For lot-specific certificates of analysis, see Section **Contact and Support**.

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

Layout changes.
Editorial changes.
Change of the constituent of Binding buffer in the Content Chapter.
Removed information related to the REACH Annex XIV.
Adapted the Result Chapter to show data derived when the new Binding Buffer is used.

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.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 L	11 666 789 001
Red Blood Cell Lysis Buffer	100 mL, 50-500 reactions, depending on sample size (1-500 µL)	11 814 389 001
Lysozyme	10 g, *Not available in US*	10 837 059 001

6.4. Trademarks

LIGHTCYCLER is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

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 **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

1123.11824376001 ⑮

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany