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High Pure RNA Isolation Kit

Version: 13
Content version: April 2017

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

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Lysis/-Binding Buffer	4.5 M guanidine-HCl, 50 mM Tris-HCl, 30% Triton X-100 (w/v), pH 6.6 (25°C)	25 ml
2		DNase I, recombinant, lyophilizate	10 KU lyophilized DNase I	Resuspend in 0.55 ml Elution Buffer
3	white	DNase Incubation Buffer	1 M NaCl, 20 mM Tris-HCl and 10 mM $MnCl_2$, pH 7.0 (25°C)	10 ml
4	black	Wash Buffer I	5 M guanidine hydrochloride and 20 mM Tris-HCl, pH 6.6 (25° C); final concentrations after addition of 20 ml absolute ethanol	33 ml (add 20 ml absolute ethanol before first use)
5	blue	Wash Buffer II	20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (25°C); final concentrations after addition of 40 ml absolute ethanol	10 ml (add 40 ml absolute ethanol before first use)
6	colorless	Elution Buffer	Water, PCR Grade	30 ml
7		High Pure Filter Tubes		One bag with 50 polypropylene tubes with two layers of glass fiber fleece, for uptake of up to 700 µl sample volume
8		Collection Tubes		One bag with 50 polypropylene tubes (2 ml)

⚠ ***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)

The High Pure RNA Isolation Kit components must be stored at +15 to +25°C. Kit components are guaranteed to be stable through the expiration date printed on the label.

⚠ ***Please note, that improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions.***

Storage Conditions (Working Solution)

Solution	Storage
DNase Solution	−15 to −25°C

ⓘ *Reconstituted DNase solution has to be stored in aliquots. Aliquots stored at −15 to −25°C are stable for 1 year.*

1.3. Additional Equipment and Reagents Required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x *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 like 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 like northern blotting, RNase protection and primer extension. Up to 24 samples can be processed simultaneously in approx. 1 hour. Thus, the purification procedure is less time consuming compared with alternative methods which require extraction with organic solutions, RNA precipitation or ultracentrifugation.

1.5. Preparation Time

Total time	Approximately 1 hour (24 samples simultaneously)

2. How to Use this Product

2.1. Before you Begin

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

⚠ ***Lysis/-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 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 Lysis/-Binding Buffer and Wash Buffer I to mix with sodium hypchlorite 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.
- 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 pipets and nuclease-free pipet 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 set-up. Sample preparation, PCR/RT-PCR set-up 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 dialog.roche.com, or upon request from the local Roche office.

Working Solution

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

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

2.2. Protocols

Isolation of Total RNA from Cultured Cells (suited for 1 × 10^6 cells)

1. Resuspend cells in 200 µl PBS.

2. Add 400 µl Lysis/-Binding Buffer (green cap) and vortex for 15 s.

3. To transfer the sample to a High Pure Filter Tube:
   – Insert one High Filter Tube in one Collection Tube.
   – Pipet entire sample into the upper reservoir of the Filter Tube (max. 700 µl)

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

5. After centrifugation:
   – Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and again combine the Filter Tube and the used Collection Tube.

6. After re-inserting the Filter Tube:
   – Pipette per sample 90 µl DNase Incubation Buffer (white cap) into a sterile reaction tube, add 10 µl DNase I, mix and pipette the solution on the glass filter fleece in the upper reservoir of the filter tube.
   – Incubate for 15 min at +15 to +25°C.

7. – Add 500 µl Wash Buffer I to the upper reservoir of the Filter Tube assembly and centrifuge 15 s at 8,000 × *g*.
   – Discard flowthrough and combine Filter Tube with the used Collection Tube.

8. – Add 500 µl Wash Buffer II (blue cap) to the upper reservoir of the Filter Tube assembly and centrifuge 15 s at 8,000 × *g*.
   – Discard flowthrough and combine Filter Tube with the used Collection Tube.

9. – Add 200 µl Wash Buffer II (blue cap) to the upper reservoir of the Filter Tube assembly and centrifuge for 2 min at maximum speed (approx. 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 – 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
    – Centrifuge the tube assembly for 1 min at 8,000 × *g*.

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

Isolation of Total RNA from Human Blood (suited for 200 – 500 µl whole blood)

⚠ ***Due to the modest content of RNA in leukocytes, it is recommended, to 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; this method is described in the following section.***

1. Add 1 ml Red Blood Cell Lysis Buffer to a sterile 1.5 ml reaction 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 min at +15 to +25° C.

   ℹ *Alternatively, manually invert the sample periodically for 10 min.*

4. Centrifuge for 5 min at 500 × *g* in a standard table top 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 min 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 from step 2.

Isolation of Total RNA from Yeast (suited for 1 × 10^8 cells)

ℹ *It is recommended to 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 – 0.15/ml (0.1 A_{600} correspond to approx. 2 × 10^6 cells.)*

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

2. Add 10 µl Lyticase (0.5 mg/ml), incubate for 15 min at 30°C.

3. Follow protocol Isolation of Total RNA from Cultured Cells from step 2.

Isolation of Total RNA from Bacteria (gram positive and gram negative) (suited for 1 x 10^9 cells)

1. Collect the sample by centrifugation at 2,000 × *g* for 5 min in a standard table top centrifuge.
   – Resuspend the pellet in 200 µl 10 mM Tris, pH 8.0.

2. Add 4 µl Lysozyme (50 mg/ml), incubate for 10 min at 37°C

3. Add 400 µl Lysis/-Binding Buffer (green cap) and mix well

4. Combine the High Pure Filter Tube and the Collection Tube and pipette the sample in the upper reservoir.

5. Centrifuge for 15 s at 8,000 × *g* in a standard table top centrifuge, discard the flowthrough and again combine the Filter Tube and the used Collection Tube.

6. Pipette 90 µl DNase Incubation Buffer (white cap) into a sterile reaction tube, add 10 µl DNase I, mix and pipette the solution in the upper reservoir of the Filter Tube.
   – Incubate for 60 min at +15 to +25°C.

7. Follow protocol Isolation of Total RNA from Cultured Cells from 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, Roche recommends using 1 to 10 µl in the RT reaction. Perform an RT-minus control reaction (by omitting addition of reverse transcriptase to the cDNA synthesis reaction). This will indicate whether your RNA sample contains residual genomic DNA by causing false-positive results.

Typical RNA yields from different samples obtained with the High Pure RNA Isolation Kit :

Source	Average yield
Cultured cells (10^6 cells) (K562)	15 µg
Whole blood, human (200 - 500 µl)	Not measurable, use in RT-PCR only
Yeast (10^8 cells) *S. cerevisiae*	20 µg
Bacteria (10^9 cells) *E. coli* *B. subtilis*	 50 µg 35 µg

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions reducing their functionality	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
	No ethanol added to Wash Buffer	Add absolute ethanol to the buffers before use.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always mark Wash Buffer I and Wash Buffer II vials to indicate whether ethanol has been added or not.
	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 Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbance ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light	① Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 min at maximum speed. ② 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 im-mediately 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

Isolation of RNA is a prerequisite for the analysis of gene expression. Frequently applied techniques like reverse transcriptase-PCR (RT-PCR), northern blotting, RNase protection and primer extension require the use of intact, undegraded RNA from different sample materials like 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, applied directly onto the glass fiber fleece.

(4) Bound nucleic acids are washed with a special Inhibitor Removal 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

1×10^6 K 562 cells are treated as described in the protocol for cultured cells. RNA yield is determined by measuring the optical density at 260 nm. At least 10 µg of total RNA are isolated. Integrity and size distribution are examined by the banding pattern of ribosomal RNA in a denaturing agarose gel. 100 ng of isolated total RNA is used in first strand synthesis with reverse transcriptase M-MuLV and $p(dT)_{15}$ as a primer. In the following PCR, accomplished with Expand High Fidelity PCR System and specific primers for glycerinaldehyde 3-phosphate dehydrogenase (G3PDH), the expected amplification product of 983 bp is obtained.
Absence of contaminating DNA is examined by a PCR without preceding RT-reaction; no amplification product is obtained; all kit components are function tested for absence of RNases according to the current Quality Control procedures.

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

Editorial changes.
Layout changes.

6.3. Ordering Information

Roche 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 lifescience.roche.com.

Product	Pack Size	Cat. No.
Reagents , kits		
Lysozyme	10 g	10 837 059 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Red Blood Cell Lysis Buffer	100 ml, for 50-500 reactions, depending on sample size (1-500 µl)	11 814 389 001

6.4. Trademarks

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6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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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.

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