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

High Pure RNA Paraffin Kit

Version: 16
Content Version: November 2020

For the isolation of total RNA from fresh-frozen and formalin-fixed, paraffin-embedded tissue

Cat. No. 03 270 289 001 1 kit
up to 100 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	white	Tissue Lysis Buffer	For the lysis of cells	▪ 20 ml
2	pink	Proteinase K, PCR grade	Lyophilizate for sample homogenization and inactivation of endogenous nucleases	▪ 2 × 90 mg
3	green	Binding Buffer	Contains 5 M guanidine-thiocyanate	▪ 2 × 80 ml
4	black	Wash Buffer I	Contains 5 M guanidine-HCl (final concentration after addition of ethanol)	▪ 100 ml, add 60 ml absolute ethanol
5	blue	Wash Buffer II		▪ 50 ml, add 200 ml absolute ethanol
6	white	DNase I	4 kU DNase I	▪ Lyophilizate for digestion of residual DNA
7	colorless	DNase Incubation Buffer, 10× conc.		▪ 2 × 1 ml
8	colorless	Elution Buffer	Water, PCR Grade	▪ 2 × 30 ml
9		High Pure Filter Tubes		▪ Four bags containing 50 polypropylene tubes with two layers of glass fiber fleece, for use of up to 800 µl sample volume
10		Collection Tubes		▪ Four bags containing 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 Plasmid Isolation Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable until the expiration date printed on the label.***

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) may lead to formation of salt precipitates in the buffers which will adversely impact the performance of the kit.***

Storage Conditions (Working Solution)

Solution	Storage
DNase I	-15 to -25°C
Proteinase K	-15 to -25°C

1.3. Additional Equipment and Reagent required

- Absolute ethanol
- Hemo-De or Xylene
- SDS, 10%
- Microcentrifuge tubes, 1.5 ml / 2.0 ml, sterile
- Homogenization device (mortar / pestle, rotor-stator homogenizer [*e.g.* Ultraturrax], or bead mill [*e.g.,* MagNA Lyser Instrument*, Fast Prep or Ribolyser])
- Standard tabletop microcentrifuge capable of 13,000 *x g* centrifugal force

1.4. Application

The High Pure RNA Paraffin Kit is designed for the isolation of total RNA from formalin-fixed, paraffin-embedded tissue as well as from fresh-frozen tissue research samples for use in RT-PCR. The quality of RNA from paraffin-sections achieved with the kit is suitable for relative quantification of mRNA with RT-PCR especially on the LightCycler® 2.0 System.

1.5. Preparation Time

Assay Time

Total time	Approx. 2 hours (without overnight incubation)

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- 5 – 10 µm sections from formalin-fixed, paraffin-embedded tissue (e.g., colon, breast, liver, kidney, spleen of mammal species incl. human research samples)
- 20 – 30 mg fresh-frozen solid tissue
- 3 × 5 µm tissue sections from fresh-frozen tissue

General Considerations

Handling Instructions

RNA in sample material is subject to degradation by intracellular RNases until it is flash frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing agents. It is therefore imperative that

- Samples are immediately flash frozen in liquid nitrogen and stored at -70°C or are processed as soon as collected.
- Frozen tissue should not be allowed to thaw during handling (e.g., weighing).
- The relevant procedures should be carried out as quickly as possible.

Samples can also be stored at -80°C in Lysis/Binding Buffer after disruption and homogenization. Yields may vary depending on storage time. During fixation in formalin intracellular RNases become inactivated. However, RNA is degraded and cross-linked to proteins or inter- or intra-molecularly. Therefore formalin-fixed, paraffin-embedded tissue can be stored and handled at +15 to +25°C.

⚠ ***Guanidine-hydrochloride in Binding Buffer and Wash Buffer I is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***

⚠ ***Do not allow these buffers to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.***

⚠ ***Never store or use the Binding Buffer near human or animal food.***

⚠ ***Always wear gloves and follow standard safety precautions when handling these buffers.***

⚠ ***Use sterile disposable polypropylene tubes and tips to avoid RNase contamination. Always wear gloves during the assay.***

⚠ ***Do not allow the Binding or Wash buffers 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.
- 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 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 dialog.roche.com, or upon request from the local Roche office.

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

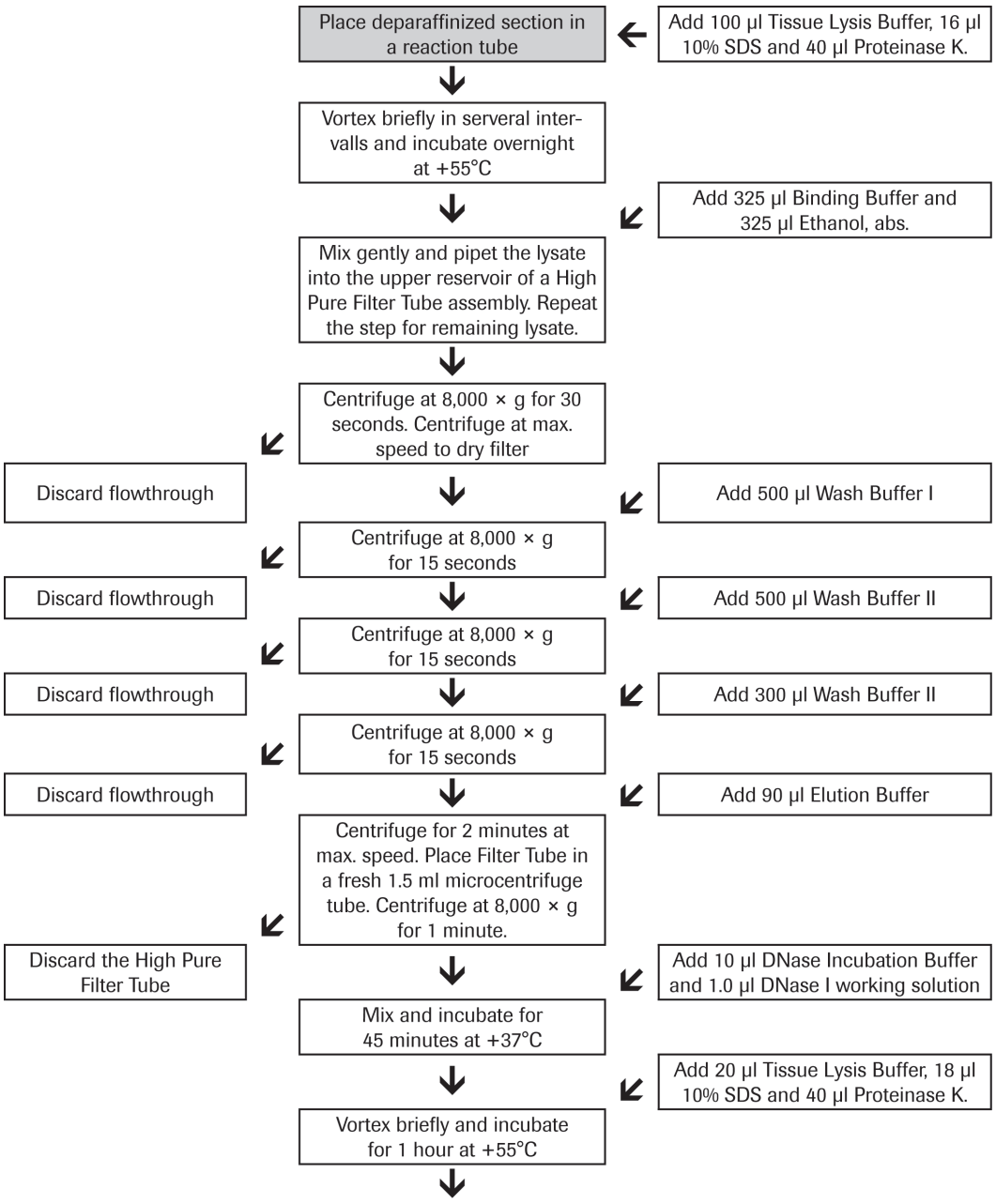
Working Solution

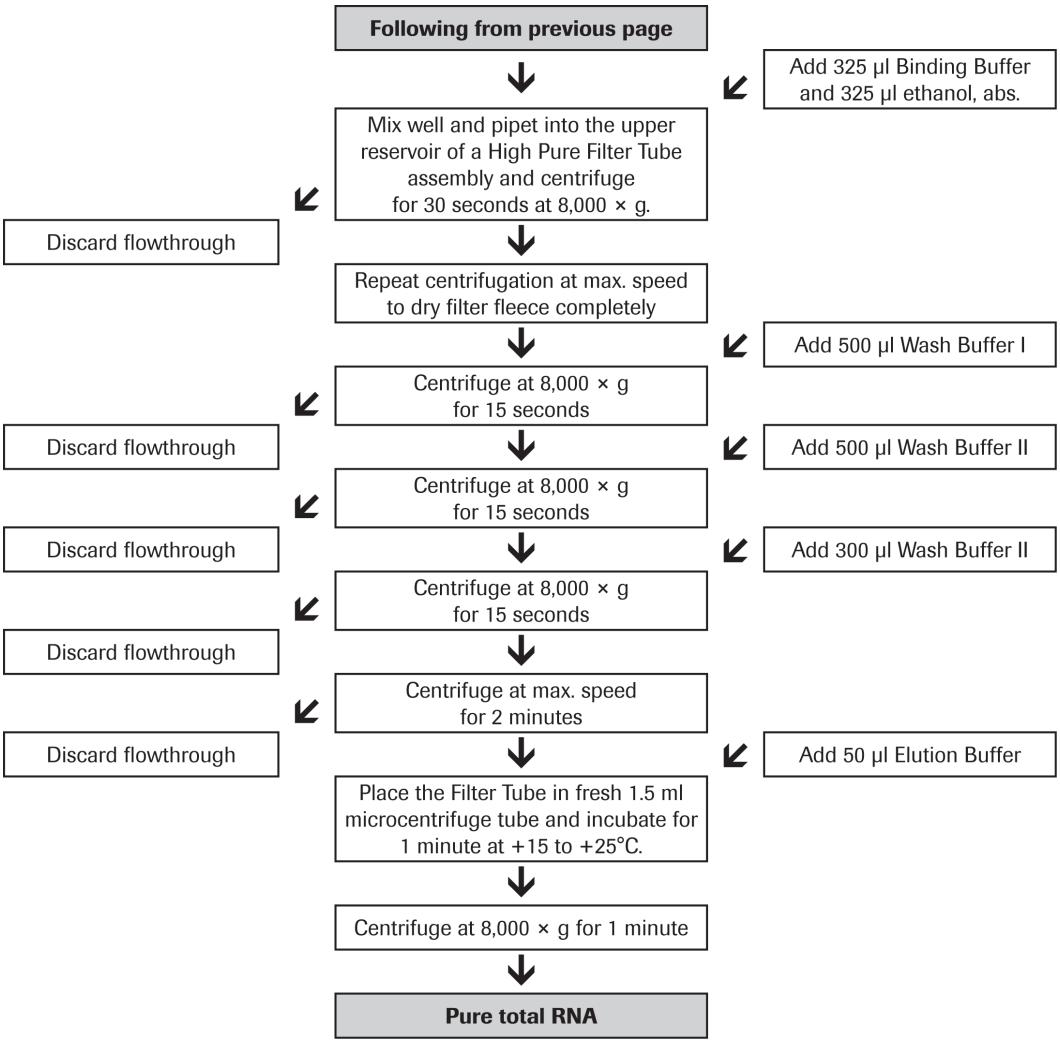
In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability	For use in...
Proteinase K (Vial 2; pink cap)	Dissolve Proteinase K in 4.5 ml Elution Buffer.	▪ Store aliquots at −15 to −25°C ▪ Stable for 12 months.	Sample lysis protocol step 1
Wash Buffer I (Vial 4; black cap)	Add 60 ml absolute ethanol to Wash Buffer. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Washing and elution protocol step 1
Wash Buffer II (Vial 5; blue cap)	Add 200 ml absolute ethanol to Wash Buffer. ⚠ ***Label and date bottle accordingly after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Washing and elution protocol step 2 and 3
DNase I (Vial 6; white cap)	Dissolve DNase I in 800 µl Elution Buffer and mix thoroughly.	▪ Store aliquots at −15 to −25°C ▪ Stable for 12 months.	Washing and elution protocol step 5

2.2. Protocols

Experimental Overview





Protocol for the Isolation of RNA from Formalin-fixed, Paraffin-Embedded Tissue

Deparaffinization

The following table describes the deparaffinization procedure for one 5 - 10 µm section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube:

1. Add 800 µl Hemo-De (or Xylene) to one 5 - 10 µm section in a 1.5 ml reaction tube.
   - Incubate for 5 minutes and mix several times overhead during incubation.

2. Add 400 µl ethanol absolute and mix.
   - Centrifuge for 2 minutes at maximum speed (12,000 - 14,000 × *g*) and discard supernatant.

3. Add 1 ml ethanol absolute and mix by overhead shaking.
   - Centrifuge for 2 minutes at maximum speed and discard supernatant.

4. Blot the tube briefly onto a paper towel to get rid of ethanol residues.
   - Dry the tissue pellet for 10 minutes at +55°C.
   - Proceed with step 1 of the RNA isolation protocol.

Alternative Procedure

The following table describes the deparaffinization procedure for one 5 - 10 µm section of formalin-fixed, paraffin-embedded tissue on a microscope slide:

1. Place the slide in a Hemo-De (or Xylene) bath.
   - Incubate for 10 minutes.

2. Tap off excess liquid.
   - Place the slide into ethanol absolute for 10 minutes.

3. Change bath and incubate the slide for additional 10 minutes in ethanol absolute

4. Scratch the deparaffinized section from the slide by using a sterile single-use scalpel and place it into a 1.5 ml reaction tube.
   ⚠ ***To avoid scattering of the tissue scratch the section from the microscope slide before it has dried.***

5. Dry the tissue for 10 minutes at +55°C.
   - Proceed with step 1 of the RNA isolation protocol.

RNA Isolation Protocol

The following protocol describes the RNA isolation from one 5 - 10 µm section of formalin-fixed, paraffin-embedded tissue.

If necessary 3 preparations can be pooled after step 4.

1. To one tissue pellet (deparaffinized as described above) add 100 µl Tissue Lysis Buffer (Bottle 1, white cap), 16 µl 10% SDS and 40 µl Proteinase K working solution.
   - Vortex briefly in several intervals and incubate overnight at +55°C.

2. Add 325 µl Binding Buffer (Bottle 3, green cap) and 325 µl ethanol absolute.
   - Mix gently by pipetting up and down.

3. Combine the High Pure Filter Tube and the collection tube and pipet the lysate into the upper reservoir.

4. Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.
   Step 3 - 4 can be repeated, in case RNA needs to be pooled, with 2 more tissue pellet preparations.

5. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

6. Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
   - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

7. Add 500 µl Wash Buffer II working solution (Bottle 5, blue cap).
   -Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

8. Add 300 µl Wash Buffer II working solution (bottle 5, blue cap)
   - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

9. Centrifuge the High Pure Filter Tube for 2 minutes at maximum speed.

10. Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube and add 90 µl Elution Buffer (Bottle 8, colorless cap).
    - Centrifuge for 1 minute at 8,000 × *g*.

11 Add 10 µl DNase Incubation Buffer, 10x (Bottle 7, colorless cap) and 1 µl DNase I working solution to the eluate and mix.
- Incubate for 45 minutes at +37°C.

12 Add 20 µl Tissue Lysis Buffer (Bottle 1, white cap), 18 µl 10% SDS and 40 µl Proteinase K working solution.
- Vortex briefly.
- Incubate for 1 hour at +55°C.

13 Add 325 µl Binding Buffer (Bottle 3, green cap) and 325 µl ethanol absolute.
- Mix and pipet into a fresh High Pure Filter Tube with collection tube.

14 Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.

15 Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

16 Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

17 Add 500 µl Wash Buffer II working solution (bottle 5, blue cap).
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

18 Add 300 µl Wash Buffer II working solution (Bottle 5, blue cap).
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

19 Centrifuge the High Pure Filter Tube for 2 minutes at maximum speed.

20 Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.
- Add 50 µl Elution Buffer (Bottle 8, colorless cap), incubate for 1 minute at +15 to +25°C.
- Centrifuge for 1 minute at 8,000 × *g* to collect the eluated RNA.

21 The microcentrifuge tube now contains the eluted RNA. Either use 10 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at -80°C for later analysis.

⚠ ***Before photometrical determination of RNA concentration, centrifuge the eluate for 2 minutes at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.***

Protocol for the Isolation of RNA from Fresh Frozen Tissue

Disruption and Homogenization

Efficient disruption and homogenization of the sample material is essential for intracellular RNA isolation procedures from tissues. The full disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields of RNA. Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears the high molecular weight genomic DNA and other high molecular weight cellular components to create a homogeneous lysate. Incomplete homogenization results in significantly reduced yields.

Disruption and Homogenization using Rotor-Stator Homogenizers

1. Add 400 µl of Tissue Lysis Buffer to the sample.

2. Homogenize according to the instrument supplier's instructions. In the presence of Lysis buffer, Rotor-Stator homogenizers thoroughly disrupt and simultaneously homogenize tissues in 5 - 90 seconds, depending on the toughness of the sample.

Foaming of the sample should be kept to a minimum by keeping the tip of the homogenizer submerged and holding the immersed tip to one side of the tube.

Disruption using a mortar and pestle

1. Freeze the sample immediately in liquid nitrogen.

2. Grind to a fine powder under liquid nitrogen.

3. Transfer the frozen tissue powder into a tube with 400 µl Tissue Lysis Buffer and mix thoroughly.

Disruption using MagNA Lyser Instrument, Fast Prep or Ribolyser

1. Add 400 µl of Tissue Lysis Buffer to the sample.

2. Homogenize according to the instrument supplier's instructions.

RNA Isolation Protocol

The following protocol describes the RNA isolation from 20 - 30 mg of fresh frozen tissue.

1. Homogenize 20 - 30 mg of fresh-frozen tissue with a suitable method, as listed above.

2. Centrifuge the lysate for 2 minutes at maximum speed in a microcentrifuge.
   - Transfer the supernatant to a fresh 2 ml reaction tube.

3. Add 1 ml Binding Buffer (Bottle 3, green cap) and 600 µl ethanol absolute.
   - Mix gently by pipetting up and down.

4. Combine the High Pure Filter Tube and the collection tube and pipet half of the volume of the lysate into the upper reservoir.

5. Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.

6. Pipet the rest of the lysate into the same High Pure Filter Tube and centrifuge for 30 seconds as described in step 5.

7. Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

8. Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
   - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

9. Add 500 µl Wash Buffer II working solution (Bottle 5, blue cap).
   - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

10. Add 300 µl Wash Buffer II working solution (Bottle 5, blue cap).

- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

⓫ Centrifuge for 2 minutes at maximum speed.

⓬ Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.
- Add 90 µl Elution Buffer (Bottle 8, colorless cap).
- Centrifuge for 1 minute at 8,000 × *g*.

⓭ Add 10 µl DNase Incubation Buffer, 10x (Bottle 7, colorless cap), 1 µl DNase I working solution to the eluate and mix.
- Incubate for 45 minutes at +37°C.

⓮ Add 20 µl Tissue Lysis Buffer (Bottle 1, white cap), 18 µl 10% SDS and 40 µl Proteinase K working solution.
- Vortex briefly.
- Incubate for 1 hour at +55°C.

⓯ Add 325 µl Binding Buffer (Bottle 3, green cap) and 325 µl ethanol absolute, mix by pipetting.
- Pipet into a fresh High Pure Filter Tube with collection tube.

⓰ Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.

⓱ Repeat the centrifugation at maximum speed in order to dry the filter fleece completely.

⓲ Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

⓳ Add 500 µl Wash Buffer II working solution (Bottle 5, blue cap).
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

⓴ Add 300 µl Wash Buffer II working solution (Bottle 5, blue cap).
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

㉑ Centrifuge the High Pure Filter Tube for 2 minutes at maximum speed.

㉒ Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.
- Add 70 µl Elution Buffer (Bottle 8, colorless cap).
- Centrifuge for 1 minute at 8,000 × *g* to collect the eluated RNA.

㉓ The microcentrifuge tube now contains the eluted RNA. Either use 10 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at -80°C for later analysis.

⚠ ***Before photometrical determination of RNA concentration, centrifuge the eluate for 2 minutes at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.***

Protocol for the RNA Isolation from Fresh Frozen Tissue Sections

The following protocol describes the RNA isolation from 3 × 5 µm sections of fresh frozen tissue:

1. To 3 × 5 µm sections of fresh-frozen tissue in a 1.5 ml reaction tube add 100 µl Tissue Lysis Buffer (Bottle 1, white cap), 16 µl 10% SDS and 40 µl Proteinase K working solution.
   - Vortex in several intervals and incubate overnight at +55°C.

2. Add 325 µl Binding Buffer (Bottle 3, green cap) and 325 µl ethanol absolute.
   - Mix gently by pipetting up and down.

3. Combine the High Pure Filter Tube and the collection tube and pipet the lysate into the upper reservoir.

4. Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.

5. Repeat the centrifugation at maximum speed to dry the filter fleece completely.

6. Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
   - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

7. Add 500 µl Wash Buffer II working solution (Bottle 5, blue cap).
   - Centrifuge for 15 sec at 8,000 × *g*, discard the flowthrough.

8. Add 300 µl Wash Buffer II working solution (bottle 5, blue cap)
   Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

9. Centrifuge the High Pure Filter Tube for 2 minutes at maximum speed.

10. Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube and add 90 µl Elution Buffer (Bottle 8, colorless cap).
    - Centrifuge for 1 minute at 8,000 × *g*.

11. Add 10 µl DNase Incubation Buffer, 10x (Bottle 7, colorless cap) and 1 µl DNase I working solution to the eluate and mix.
    - Incubate for 45 minutes at +37°C.

12. Add 20 µl Tissue Lysis Buffer (Bottle 1, white cap), 18 µl 10% SDS and 40 µl Proteinase K working solution.
    - Vortex briefly.
    - Incubate for 1 hour at +55°C.

13. Add 325 µl Binding Buffer (Bottle 3, green cap) and 325 µl ethanol absolute.
    - Mix and pipet into a fresh High Pure Filter Tube with collection tube.

14. Centrifuge for 30 seconds at 8,000 × *g* in a microcentrifuge and discard the flowthrough.

15. Repeat the centrifugation at maximum speed to dry the filter fleece completely.

16. Add 500 µl Wash Buffer I working solution (Bottle 4, black cap) to the upper reservoir.
    - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

17. Add 500 µl Wash Buffer II working solution (bottle 5, blue cap).
    - Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

18 Add 300 µl Wash Buffer II working solution (Bottle 5, blue cap).
- Centrifuge for 15 seconds at 8,000 × *g*, discard the flowthrough.

19 Centrifuge the High Pure Filter Tube for 2 minutes at maximum speed.

20 Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.
- Add 70 µl Elution Buffer (Bottle 8, colorless cap).
- Centrifuge for 1 minute at 8,000 × *g* to collect the eluated RNA.

21 The microcentrifuge tube now contains the eluted RNA. Either use 10 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at -80°C for later analysis.

⚠ ***Before photometrical determination of RNA concentration, centrifuge the eluate for 2 minutes at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.***

3. Results

Purity

Purified RNA is free of DNA, nucleases and all cellular and sample components that interfere with RT-PCR according to the current Quality Control Procedures.
Typically RNA fragments isolated from formalin-fixed tissue ranged from 150 up to 1,500 bases. The bulk of RNA is 250 bases in length.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low RNA 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 condi-tions 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 freedom from contamination.
		After any lyophilized reagent is constituted, aliquot it and store the aliquot at -15 to -25°C.
	Ethanol not added to Wash Buffer	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always mark Wash Buffer vial and Inhibitory Removal Buffer vial 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.
	Tissue stored and handled in less than optimal conditions	Use fresh tissue and disrupt immediately or flash frozen tissue stored at -70° C or below. Frozen tissue should not be allowed to thaw during handling prior to disruption in Lysis/Binding Buffer.
	Ethanol not added to the lysate in step 3	Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.
	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.
Tissue homogenate is viscous and difficult to pipet, low RNA yield	Insufficient disruption or homogenization	Add 350 ml of Lysis / Binding Buffer and repeat homogenization step to reduce viscosity.
	Too much starting material	Reduce amount of starting material and / or increase the amount of Lysis / Binding Buffer.
Clogged Filter Tube	Insufficient disruption and / or homogenization	Increase the disruption time for the Rotor-Stator homogenizer or pass through syringe /needle additional times.
	Too much starting material	Reduce amount of starting material and / or increase the amount of Lysis / Binding Buffer.
A_{260} nm value of eluate too high	Glass fibers, which might 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.
Samples “pop” out of wells in agarose gels	Eluate contains ethanol (from the Wash Buffer)	After the wash step, do not let the flowthrough touch the bottom of the High Pure Filter Tube.
		Empty collection tube, reinsert the Filter Tube in emptied collection tube, and recentrifuge for 30 seconds.

5. Additional Information on this Product

5.1. Test Principle

Tissue samples are disrupted and homogenized during an overnight incubation with Proteinase K (paraffin samples) or by using a suitable tissue homogenizer (fresh-frozen tissue). Nucleic acids (NA) bind in the presence of a chaotropic salt specifically to the surface of glass fibers pre-packed in the High Pure Purification Filter Tube. The binding reaction occurs within seconds due to the disruption of the organized structure of water molecules and the interaction with nucleic acids. The binding process is specific for nucleic acids in general, but the binding conditions are optimized for RNA. Bound RNA is purified in a series of rapid "wash-and-spin" steps to remove cellular components. After elution from the column, residual DNA is digested by incubating the eluate with DNase I. A second incubation step with Proteinase K improves the purity of RNA. Finally, a low salt elution releases the RNA from the glass fiber. The process does not require RNA precipitation, or organic solvent extractions.

① Samples are disrupted in Tissue Lysis Buffer and homogenized.

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

③ Bound nucleic acids are washed, thereby purified from salts, proteins, or other impurities.

④ Elution of the purified nucleic acids with Elution Buffer is followed by incubations with DNase I and Proteinase K to improve purity.

⑤ Second round of binding RNA to glass fiber fleece and washing steps.

⑥ Final elution of highly pure RNA.

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.
New information added related to the REACH Annex XIV.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Instruments		
MagNA Lyser Instrument	1 instrument, 120 V	03 358 968 001
	1 instrument, 230 V, *Not available in US*	03 358 976 001

6.4. Trademarks

MAGNA LYSER 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:
List of biochemical reagent products.

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

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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