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

High Pure miRNA Isolation Kit

Version: 09
Content Version: November 2020

For purification of small RNA or total RNA from animal cells, tissue, or FFPE sections

Cat. No. 05 080 576 001 1 kit
50 miRNA isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial	Cap	Label	Function / Description	Content
1	white	Paraffin Tissue Lysis Buffer	▪ For FFPE[1] sections only	20 ml
2	pink	Proteinase K	▪ Lyophilizate ▪ For FFPE[1] sections only	100 mg
3	green	Binding Buffer	▪ Contains guanidine thiocyanate	80 ml
4	colorless	Binding Enhancer		20 ml
5	blue	Wash Buffer		2 × 10 ml, add 40 ml absolute ethanol to each vial
6	colorless	Elution Buffer	▪ Water, PCR Grade	30 ml
7		High Pure Filter Tubes	▪ for processing up to 700 µl sample volume	2 bags with 50 columns
8		Collection Tubes		2 bags with 50 polypropylene tubes (2 ml).

(1) FFPE formalin-fixed, paraffin-embedded

⚠ ***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*

⚠ ***Do not use vessels or pipettes containing polystyrene (PS) when working with the Binding Enhancer (Vial 4).***

1.2. Storage and Stability

Storage Conditions (Product)

⚠ ***The High Pure miRNA 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
Proteinase K	-15 to -25°C

ⓘ *After dissolving Proteinase K lyophilizate in Elution Buffer, aliquot and store the solutions at -15 to -25°C. The solutions are stable for 12 months.*

1.3. Additional Equipment and Reagent required

Refer to the list below for additional reagents and equipment required for the clean up of small RNA:

- Absolute ethanol
- 10 % SDS solution (for FFPE sections only).
- Hemo-De or Xylene (for FFPE sections only).
- Standard tabletop microcentrifuge capable of 13,000 × g centrifugal force
- Microcentrifuge tubes, 1.5 ml / 2.0 ml, sterile
- Mortar and Pestle, MagNA Lyser Instrument or Rotor-Stator Homogenization device (*e.g.,* Ultra Turrax)
- Water, PCR grade*
- Optional: DNase I rec., RNase-free*
- Optional: MagNA Lyser Green Beads*

1.4. Application

The kit is designed for the isolation of small RNA (e.g., miRNA / microRNA) from animal cells, tissue samples, or formalin-fixed, paraffin-embedded tissue. It can be used to purify total RNA or to prepare samples enriched for small RNA <100 nucleotides.
The quality of the preparation is suitable for cloning, northern blotting, miRNA array hybridization, and for the relative quantification of miRNA with RT-PCR (e.g., on the LightCycler® 480 System).

1.5. Preparation Time

Assay Time

Total time	Approx. 30 minutes (without Proteinase K incubation and deparaffinization)

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Samples can be derived from:
- Animal tissue 1 - 50 mg either fresh deep frozen or stored in "RNAlater®"
- Plant tissue 1 - 100 mg
- Animal cell culture up to 10^6 cells
- Paraffin-embedded tissue 5 - 10 µm sections
- Liquid samples up to 150 µl: from cytoplasmic extracts or enzymatic reactions (*e.g.,* DNase digestion, RNA labeling, RNA ligations, *In vitro* transcriptions)

Sample Material	Disruption / homogenization method of choice	Suggested procedure
Animal tissue	Rotor Stator Homogenizer or MagNA Lyser Instrument	▪ Add 400 µl 20% Binding Buffer in a sterile tube, or a MagNA Lyser Green Beads tube
		▪ Then add 1 - 50 mg of tissue and homogenize. Centrifuge lysate for 2 minutes at maximum speed in a microcentrifuge and transfer the collected supernatant into a new sterile tube for Step 1 of RNA isolation.
Animal cell culture suspension cells	Mix by pipetting up and down, homogenize with syringe, if necessary.	▪ Spin down cells at low centrifugal force (200 × g) at +15 to +25°C for 10 minutes and remove supernatant.
		▪ Then lyse cells by adding 150 µl of 20% Binding Buffer and homogenize by pipetting up and down until lysate appears clear.
		▪ Pass the lysate 5 - 10 times through a 20-gauge needle (0.9 mm) attached to a sterile plastic syringe if the solution is not homogeneous after this step.
		▪ Centrifuge lysate for 2 minutes at maximum speed in a microcentrifuge and transfer the collected supernatant into a new sterile tube for Step 1 of RNA isolation.
Animal cell culture adherent cells	Mix by pipetting up and down, homogenize with syringe, if necessary.	▪ Remove culture supernatant with a pipet.
		▪ Then lyse cells by adding 150 µl of 20% Binding Buffer and homogenize by pipetting up and down until lysate appears clear.
		▪ Pass the lysate 5 - 10 times through a 20-gauge needle (0.9 mm) attached to a sterile plastic syringe if the solution is not homogeneous after this step.
		▪ Centrifuge lysate for 2 minutes at maximum speed in a microcentrifuge and transfer the collected supernatant into a new sterile tube for Step 1 of RNA isolation. *Alternatively, cells can be trypsinized and washed with PBS and then treated like suspension cells (see above).*

Plant tissue	Mortar and pestle followed by homogenization	▪ Freeze the 1 – 100 mg of sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen.
		▪ Transfer the frozen tissue powder into a sterile tube, or a MagNA Lyser Green Beads tube con-taining 400 ml of 20% Binding Buffer.
		▪ Measure the amount of tissue using pre-weighed sterile caps at this step, if needed.
		▪ Continue as quickly as possible with the homogenization step using a syringe and needle or the MagNA Lyser Instrument.
		▪ Centrifuge lysate for 2 minutes at maximum speed in a microcentrifuge and transfer the collected supernatant into a new sterile tube for Step 1 of RNA isolation.
Liquid samples	Mix by pipetting up and down.	▪ Up to 150 µl of liquid can be directly used for step 1 of RNA isolation.
Formalin-fixed, paraffin-embedded tissue	Deparaffinize, then protease treatment	▪ To one 5 – 10 µm section (1 cm × 1 cm) in a 1.5 ml reaction tube, add 800 µl xylene, incubate for 5 minutes, and mix by overhead shaking.
		▪ Add 400 µl ethanol abs. and mix. Centrifuge for 2 minutes at maximum speed and discard supernatant.
		▪ Add 1 ml ethanol abs. and mix by overhead shaking. Centrifuge for 2 minutes at maximum speed and discard supernatant.
		▪ Invert tube and blot briefly on a paper towel to get rid of residual ethanol. Dry the tissue pellet for 10 minutes at 55°C.
		▪ Proceed with Step 1 of RNA isolation from formalin-fixed, paraffin-embedded tissue.

Disruption and Homogenization

Efficient disruption and homogenization of the starting material is essential for intra-cellular RNA isolation procedures from tissues.
The full disruption of cell walls, extracellular material and plasma membranes of cells and organelles is absolutely essential to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields. 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. Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step.

Disruption and Homogenization using Rotor-Stator Homogenizers

In the presence of 20% Binding Buffer, rotor stator homogenizers thoroughly disrupt and simultaneously homogenize tissues in 5 to 90 seconds, depending on the viscosity of the sample. By a combination of turbulence and mechanical shearing, the sample will be disrupted and homogenized.
Keep foaming of the sample to a minimum by keeping the tip of the homogenizer submerged and holding the immersed tip on the side of the tube.

Disruption using a Mortar and Pestle

⚠ ***This step is for disruption only. Homogenization must be performed separately!***
For disruption using mortar and pestle, freeze the sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the frozen tissue powder into a tube with 20% Binding Buffer. Measure the amount of tissue using pre-weighed sterile caps at this step, if needed. Continue as quickly as possible with the homogenization step using a syringe and needle or the MagNA Lyser Instrument.

Homogenization using a Syringe and Needle

After disruption, the tissue lysate can be homogenized using a syringe and needle. High molecular weight DNA is sheared by passing the lysate through a 20-gauge needle (Φ0.9 mm) attached to a sterile plastic syringe. Pass the lysate through the needle at least 5 - 10 times, or until a homogeneous lysate is achieved.

Homogenization using the MagNA Lyser Instrument

Add 400 µl 20% Binding Buffer to a MagNA Lyser Green Beads tube. Then transfer tissue sample into the tube. Set up the MagNA Lyser Instrument as described in the Operator's Manual. Start the disruption cycle, applying speed and time settings appropriate for your special material. Refer to the Instructions for Use for details.

As an initial starting point, use the values given for some exemplary sample materials in the table below:

Sample material	Speed	Time
Plant / Food	5,000 rpm	60 s
Liver / Kidney	6,500 rpm	50 s
Spleen / Tumor Tissue	6,500 rpm	2 × 50 s[1]
Tail / Ear / Skin	6,500 rpm	2 - 3 × 50 s[1]

(1) For optimal homogenization, cool samples in the MagNA Lyser Rotor Cooling Block (approx. 30 s, +2 to +8°C) and centrifuge for 1 min at 13,000 × g to reduce foam between the disruption cycles.

General Considerations

Handling Instructions

Samples can also be stored at -80°C in 20% Binding Buffer after disruption and homogenization. Yields may vary depending on storage time. Intracellular RNases become inactivated during fixation. Formalin-fixed, paraffin-embedded tissue can be stored and handled at room temperature.

Formalin-fixation might cause RNA degradation and cross-linking to proteins.

It is recommended to use sterile disposable polypropylene tubes and tips in order to avoid RNase contamination. Wear gloves during the assay.

Guanidine-thiocyanate in Binding Buffer is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.

Do not allow Binding buffer 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.

Do not allow the Binding Buffer to mix with sodium hypochlorite found in commercial bleach solutions. The mixture can produce a highly toxic gas.

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

Samples must be immediately flash frozen in liquid nitrogen and stored at -60°C or below or must be processed as soon as collected.

Frozen tissue should not be allowed to thaw during handling.

All relevant procedures should be carried out as quickly as possible.

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

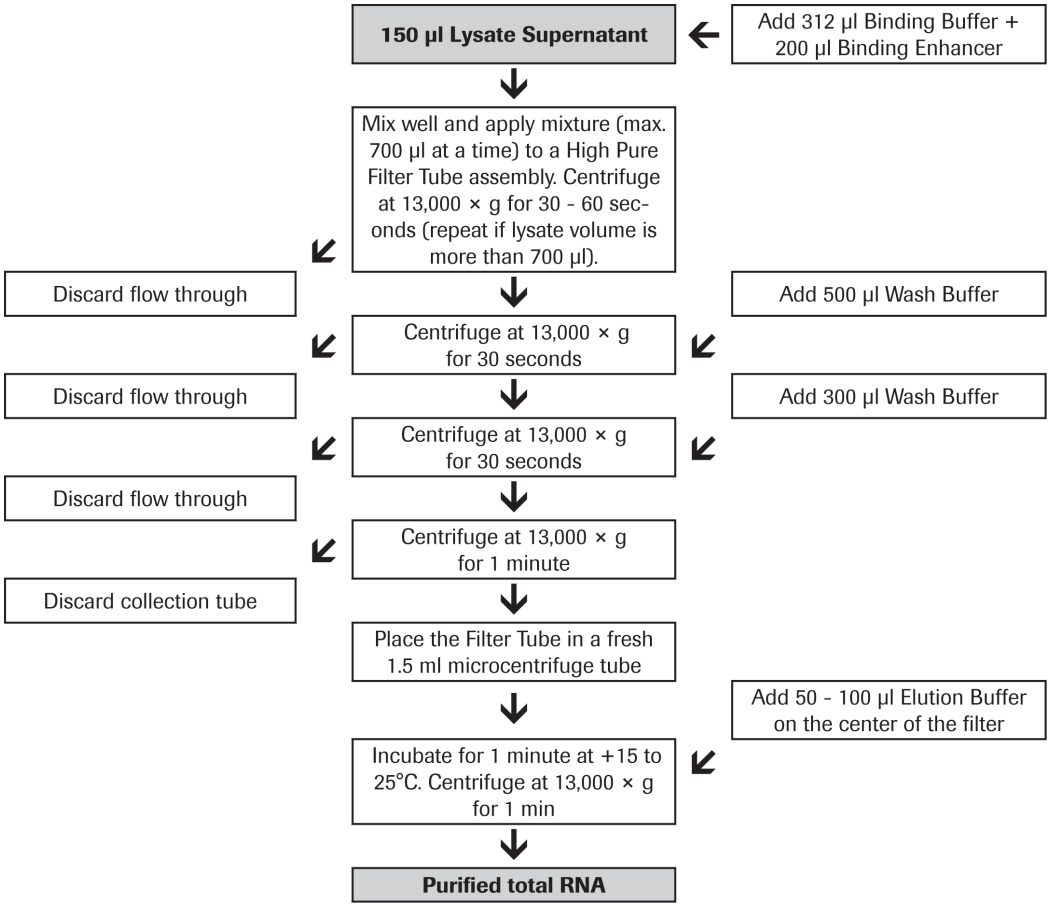
In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

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.	Tissue digestion for paraffin- embedded samples
Wash Buffer (Vial 5; blue cap)	Add 40 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.	Small RNA purification: removal of large polynucleotides, salts, and proteins
20% Binding Buffer	For one sample, mix 80 µl of Binding Buffer and 320 µl of nuclease-free, sterile, double-distilled water (or Elution Buffer) in a sterile RNase-free tube to prepare 20% Binding Buffer. For the total of 50 samples, mix 4 ml of Binding Buffer and 16 ml of nuclease-free, sterile, double-distilled water (or Elution Buffer) in a sterile RNase-free bottle to prepare 20% Binding Buffer.	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Tissue and cell disruption

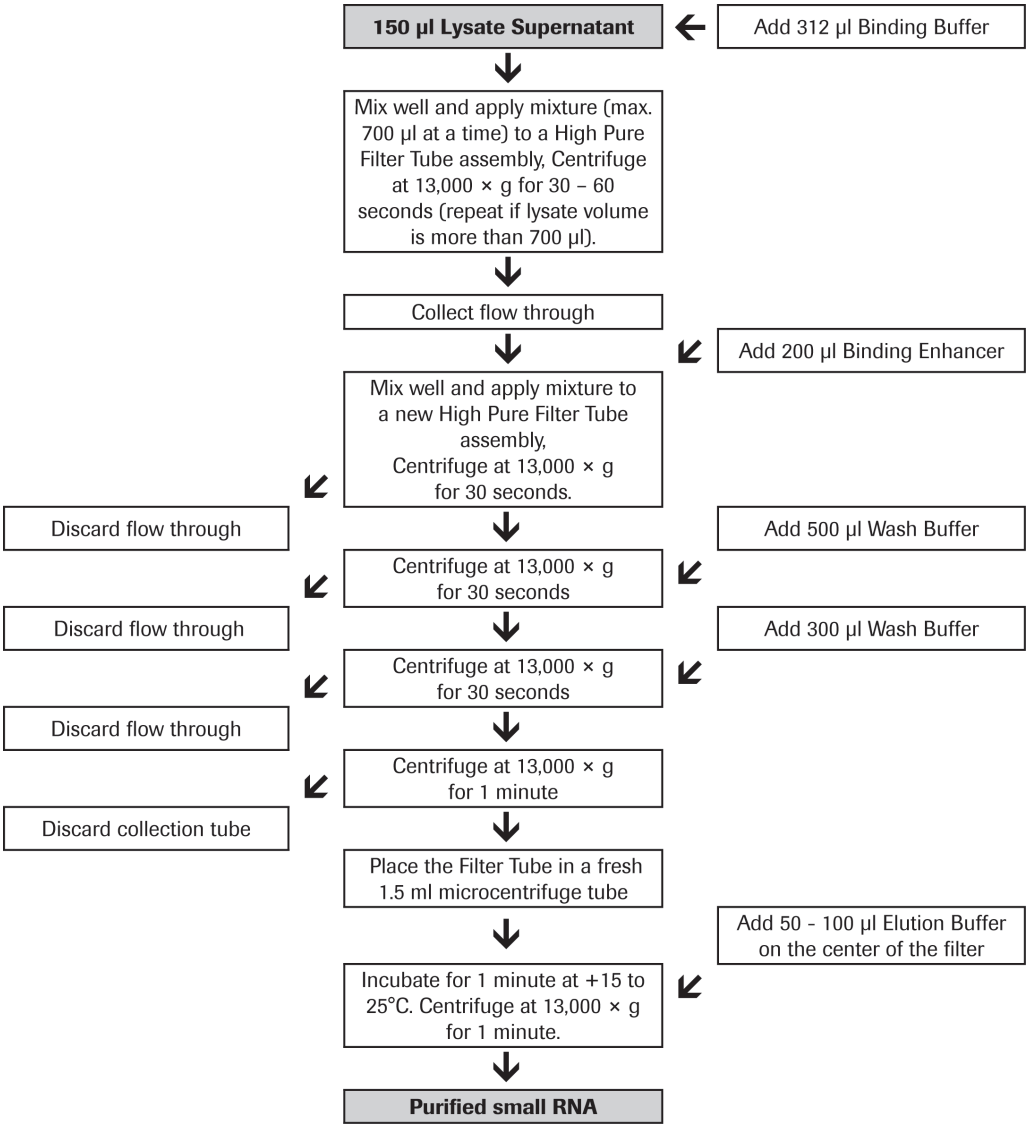
2.2. Protocols

Experimental Overview

1-Column protocol for the isolation of total RNA containing small RNA



2-Column protocol for the isolation of small RNA , 100 nucleotides



Protocols for the Isolation of microRNA from Tissue

One-Column-Protocol (total RNA)

1. Take 150 µl of cell lysate.
   For optimum results, do not add lysate from more than 10 mg of animal tissue, 50 mg of plant tissue, or 10^6 animal or plant cells to the column at this step.

2. Add 312 µl Binding Buffer, vortex briefly, then add 200 µl Binding Enhancer.
   - Vortex 3 × 5 seconds.

3. Combine the High Pure Filter Tube with a collection tube and pipet the whole mixture from Step 2 into the upper reservoir.

4. Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.
   - Step 3 - 4 can be repeated to load the column with additional sample material (do not overload the column).

5. Add 500 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

6. Add 300 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

7. Centrifuge at 13,000 × *g* for 1 minute to dry the filter fleece completely.

8. Place the High Pure Filter Tube into a fresh 1.5 ml microcentrifuge tube, add 100 µl Elution Buffer (Bottle 6, colorless cap), and incubate for 1 minute at +15 to +25°C.
   To increase RNA concentration of your sample, elution with 50 µl Elution Buffer is possible. An elution step with 100 µl Elution Buffer will increase the total yield by approx. 10%.
   - Centrifuge for 1 minute at approximately 13,000 × *g*.

9. The microcentrifuge tube now contains the eluted total RNA. Either use 0.5 - 5 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at -60 to -80°C for later analysis.

Glass fibres in the eluate may interfere with optical density measurement. Before determining the RNA concentration photometrically, centrifuge and transfer supernatant to a fresh 1.5 ml reaction tube.

Two-Column-Protocol (purified small RNA)

1. Take 150 µl of cell lysate.
   For optimum results, do not add lysate from more than 10 mg of animal tissue, 50 mg of plant tissue, or 10^6 animal or plant cells to the column at this step.

2. Add 312 µl Binding Buffer.
   - Vortex 3 × 5 seconds.

3. Combine the High Pure Filter Tube with a 2 ml collection tube and pipet the lysate into the upper reservoir.
   To minimize pipetting steps, a 2 ml microcentrifuge tube can be used instead of the collection tube.

4. Centrifuge for 30 seconds at 13,000 × *g* in a microcentrifuge and collect the flow through.

5. Add 200 µl Binding Enhancer.
   - Vortex 3 × 5 seconds.

6. Combine a new High Pure filter tube with a collection tube and pipet the whole mixture from Step 5 into the upper reservoir.

7. Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.
   - Steps 3 - 7 can be repeated to load the column with additional sample material (do not overload the column).

8. Add 500 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

9. Add 300 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

10. Centrifuge at 13,000 × g for 1 minute to dry the filter fleece completely.

11. Place the High Pure Filter Tube into a fresh 1.5 ml microcentrifuge tube, add 100 µl Elution Buffer (Bottle 6, colorless cap) and incubate for 1 minute at +15 to +25°C.
    To increase RNA concentration of your sample, elution with 50 µl -Elution Buffer is possible. An elution step with 100 µl Elution Buffer will increase the total yield by approx. 30%.
    - Centrifuge for 1 minute at 13,000 × *g*.

12 The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 - 5 µl of the eluted RNA directly in RT-PCR or store the eluted microRNA at -60 to -80°C for later analysis.

Protocol for the Isolation of microRNA from Formalin-Fixed, Paraffin-Embedded Tissue

1 Add 100 µl Paraffin Tissue Lysis Buffer, 16 µl 10% SDS, and 40 µl Pro-teinase K working solution to each deparaffinized sample as described above.
- Vortex 3 × 5 seconds.
- To increase yield, incubate at 55°C for at least 3 hours (up to over-night).

2 Add 312 µl Binding Buffer.
- Vortex briefly.

3 Add 120 µl Binding Enhancer.
- Vortex 3 × 5 seconds.

4 Combine the High Pure Filter Tube with a 2 ml collection tube and pipet the lysate into the upper reservoir.
To minimize pipetting steps a 2 ml microcentrifuge tube can be used instead of the collection tube.

5 Centrifuge for 30 seconds at 13,000 × *g* in a microcentrifuge and collect the flow through.

6 Add 205 µl Binding Enhancer.
- Vortex 3 × 5 seconds.

7 Combine a new High Pure Filter Tube with a collection tube and pipet the whole mixture from Step 6 into the upper reservoir.

8 Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.
- Steps 7 - 8 can be repeated to load the column with additional sample material (do not overload the column).

9 Add 500 µl Wash Buffer working solution.
- Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

10 Add 300 µl Wash Buffer working solution.
- Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

11 Centrifuge at 13,000 × *g* for 1 minute to dry the filter fleece completely.

12 Place the High Pure Filter Tube into a fresh 1.5 ml microcentrifuge tube, add 100 µl Elution Buffer and incubate for 1 minute at +15 to +25°C.
To increase RNA concentration of your sample, elution with 50 ml Elution Buffer is possible.
- Centrifuge for 1 minute at 13,000 × *g*.

13 The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 - 5 µl of the eluted RNA directly in RT-PCR or store the eluted microRNA at -60 to -80°C for later analysis.

It is possible to modify this protocol so it uses only 1 column; however this may lead to higher DNA contamination. For the one-column purification, use 325 µl of Binding Enhancer in Step 6 and skip Steps 3 - 5.

Protocol for the Optional Treatment of the Eluate with DNase

1. For DNase treatment, it is recommended to elute the sample with 100 µl Elution Buffer to minimize loss of sample material.
   - To approx. 100 µl, add 56 µl DNase Solution.
   - Vortex 3 × 5 seconds.
   - Incubate for 30 minutes at 37°C.

   DNase Solution: for 70 µl, mix 49.5 µl water, PCR grade, 19.5 µl 10× DNase Incubation buffer, and 1 µl DNase 1 (10 U/µl)

   DNase treatment on the column is not recommended as miRNA will elute at low-salt concentrations.

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2. Add 325 µl Binding Buffer.
   - Vortex briefly.

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3. Add 210 µl Binding Enhancer.
   - Vortex 3 × 5 seconds.

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4. Combine the High Pure Filter Tube with a 2 ml collection tube and pipet the DNase treated sample into the upper reservoir.

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5. Centrifuge for 30 seconds at 13,000 × *g* in a microcentrifuge and discard the flow through.

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6. Add 500 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

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7. Add 300 µl Wash Buffer working solution.
   - Centrifuge for 30 seconds at 13,000 × *g*, discard the flow through.

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8. Centrifuge at 13,000 × *g* for 1 minute to dry the filter fleece completely.

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9. Place the High Pure Filter Tube into a fresh 1.5 ml microcentrifuge tube, add 100 µl Elution Buffer and incubate for 1 minute at +15 to +25°C.

   To increase RNA concentration of your sample, elution with 50 µl Elution Buffer is possible. An elution step with 100 µl Elution Buffer will increase the total yield by approx. 10%.

   - Centrifuge for 1 minute at 13,000 × g.

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10. The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 - 5 µl of the eluted RNA directly in RT-PCR or store the eluted microRNA at -60 to -80°C for later analysis.

Glass fibers in the eluate may interfere with optical density measurement. Before determining the RNA concentration photometrically, centrifuge and transfer supernatant to a fresh 1.5 ml reaction tube.

3. Results

Recovery

The amount of RNA recovered is dependent on the amount of RNA applied to the glass fiber fleece, the elution volume, and the nature of the tissue. When lysate from 1 – 10 mg mouse liver tissue or 10^6 K562 cells is applied to the High Pure Filter Tube, approximately 60 – 90% of spiked miRNA is recovered using the two-column and the one-column protocol respectively. PCR amplification of the purified, eluted RNA in a LightCycler® 480 Instrument (miRNA16 kit from ABI) produces no signal without reverse transcription (No-RT-control).

Purity

- Small RNA purified by the two-column protocol is free of DNA, nucleases and all cellular and sample contaminants that interfere with RT-PCR.
- The absence of contaminating DNA is examined by PCR without a preceding RT-reaction; no amplification product is obtained.
- The integrity and size distribution of total RNA and small RNA(two-column protocol) purified with the High Pure miRNA Isolation Kit have been verified electrophoretically on a denaturing gel (15% acrylamide/TBE/urea). A 1 µg sample of miRNA 145 was spiked into the sample (before purification). Nucleic acids were visualized by ethidium bromide staining (Figure 1). In addition, electropherograms were recorded on an Agilent Bioanalyzer (Figures 2 – 4).

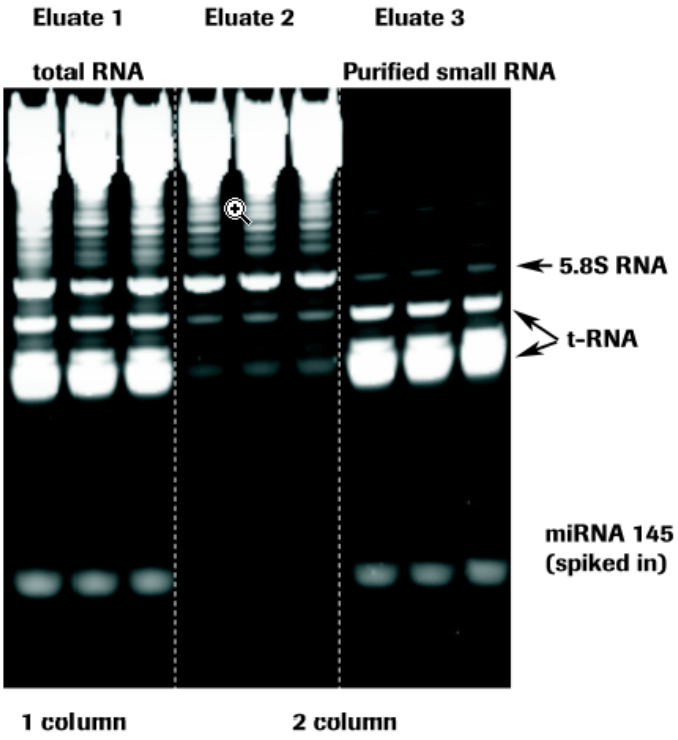
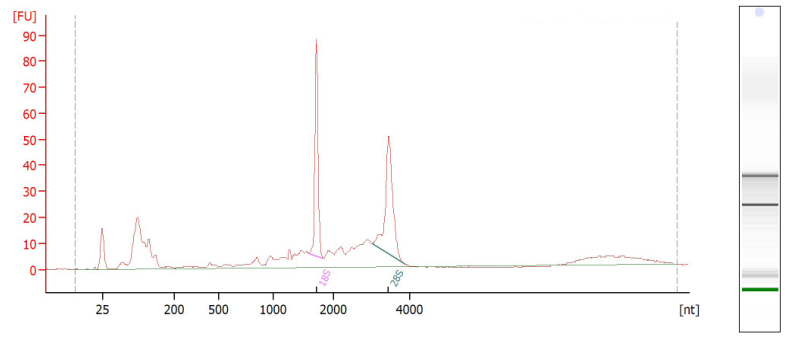
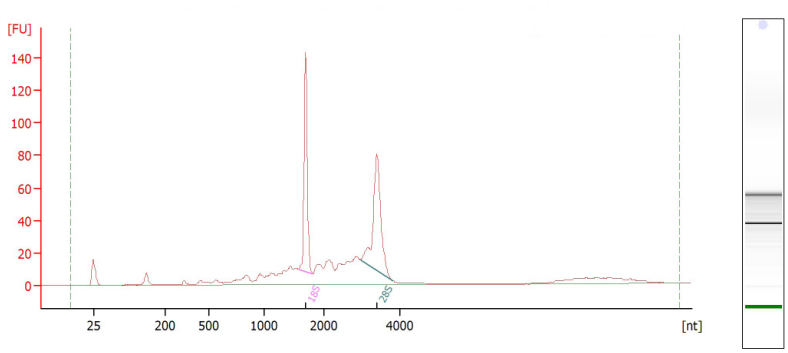


Fig. 1: Total RNA and purified small RNA
(Eluate volume: 100µl; 10µl sample per lane)
Eluate 1: One-column protocol; total RNA
Eluate 2: Two-column protocol; high molecular weight RNA eluted from the first filter tube (control of purification)
Eluate 3: Two-column protocol; purified small RNA (miRNA enriched)

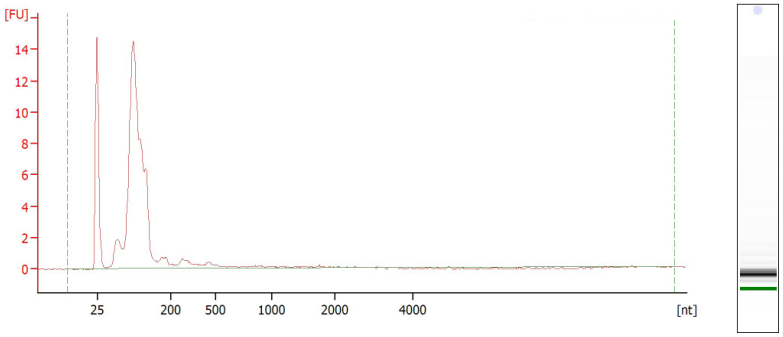
Total RNA and purified small RNA



One-column protocol; total RNA from 5 mg liver (mouse) stabilized in RNAlater®



Two-column protocol; high molecular weight RNA eluated from the first filter tube (control of purification) from 5 mg liver (mouse) stabilized in RNAlater®



Two-column protocol; purified small RNA (miRNA enriched) from 5 mg liver (mouse) stabilized in RNAlater®

Fig. 2: Total RNA and purified small RNA

Expected Yield

The concentration and purity of total RNA can be determined by measuring the absorbance at 260 nm and 280 nm in a spectrophotometer. The concentration of purified small RNA: An absorbance of 1 unit at 260 nm corresponds to 40 µg RNA per ml. The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of RNA purity and should be 2.0 or higher (RNA diluted in 20 mM Tris-HCl, pH 7.5).

Type of tissue	Yield of total RNA (one-column protocol) [µg/mg]
Mouse liver	1.5 - 9
Mouse kidney	0.5 - 7
Rat liver	1.5 - 8
Rat brain	0.5 - 2
Rat muscle	0.5 - 3.5
Rat heart	0.5 - 2.5
K562 cells	15 - 30 µg per 10^6cells

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 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 freedom from contamination.
		Store reconstituted Proteinase K aliquoted at -15 to -25°C.
	Ethanol not added to Wash Buffer	Add absolute ethanol to the Wash Buffer before use.
		After adding ethanol, mix the Wash Buffer well and store at +15 to +25°C.
		Always mark Wash 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.
	Binding Enhancer not added to the lysate	Addition of Binding Enhancer 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 sample immediately or store at -60 to -80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store immediately at -60 to -80°C.
Tissue homogenate is viscous and difficult to pipet, low RNA yield, clogged filter tube	Insufficient disruption or homogenization.	Add more 20% Binding Buffer and repeat the homogenization step to reduce viscosity.
	Too much sample material.	Reduce the amount of sample material and/or increase the amount of 20% Binding Buffer.
DNA contamination	Insufficient homogenization, too much sample material.	Perform two-column protocol or DNase treatment.
A_{260} reading of product too high	Product (column eluate) contaminated with glass fibers, which scatter light.	Centrifuge the eluate for 2 minutes at maximum speed.
		Transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.
Purified RNA sample cannot easily be loaded into the well of an agarose gel, but instead “pops out” of the well as it is loaded	Eluate containing the purified RNA product is contaminated with ethanol from the Wash Buffer.	After the last wash step, make certain flow through solution containing Wash Buffer does not contact the bottom of the High Pure Filter Tube.
		If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and recentrifuge for 30 seconds.
	Incomplete elution.	Elute RNA with two volumes of Elution Buffer (50 µl each): Be sure to centrifuge after each addition of Elution Buffer.
Concentration of RNA in the eluate is too low	Low amounts of RNA were added to the High Pure Filter Tube (in Step 1).	Decrease the volume of Elution Buffer used to recover RNA.
		Do not use less than 50 µl Elution Buffer.

5. Additional Information on this Product

5.1. Test Principle

In the presence of the chaotropic salt guanidine thiocyanate, RNA binds selectively to special glass fibers pre-packed in the High Pure Filter Tube. Bound RNA is purified in a series of rapid wash-and-spin steps to remove contaminating salts, proteins, and other cellular impurities, and then eluted using a low-salt solution. This simple method eliminates the need for organic solvent extractions and RNA precipitation, allowing for rapid purification of many samples simultaneously.
By lowering the concentration of the binding enhancer during the binding step in the two-column protocol, the small RNA containing miRNA passes the first column unbound. When the concentration of the binding enhancer is increased again, the small RNA fraction can be bound to a second High Pure Filter Tube.

Test Principle

① Sample is disrupted in 20% Binding Buffer.

② RNA is isolated by binding to the glass fibers pre-packed in the High Pure Filter Tube.

③ Bound RNA is washed to remove salts, proteins, and other cellular impurities.

④ Purified 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.
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
Reagents, kits		
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001

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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