

High Pure RNA Tissue Kit

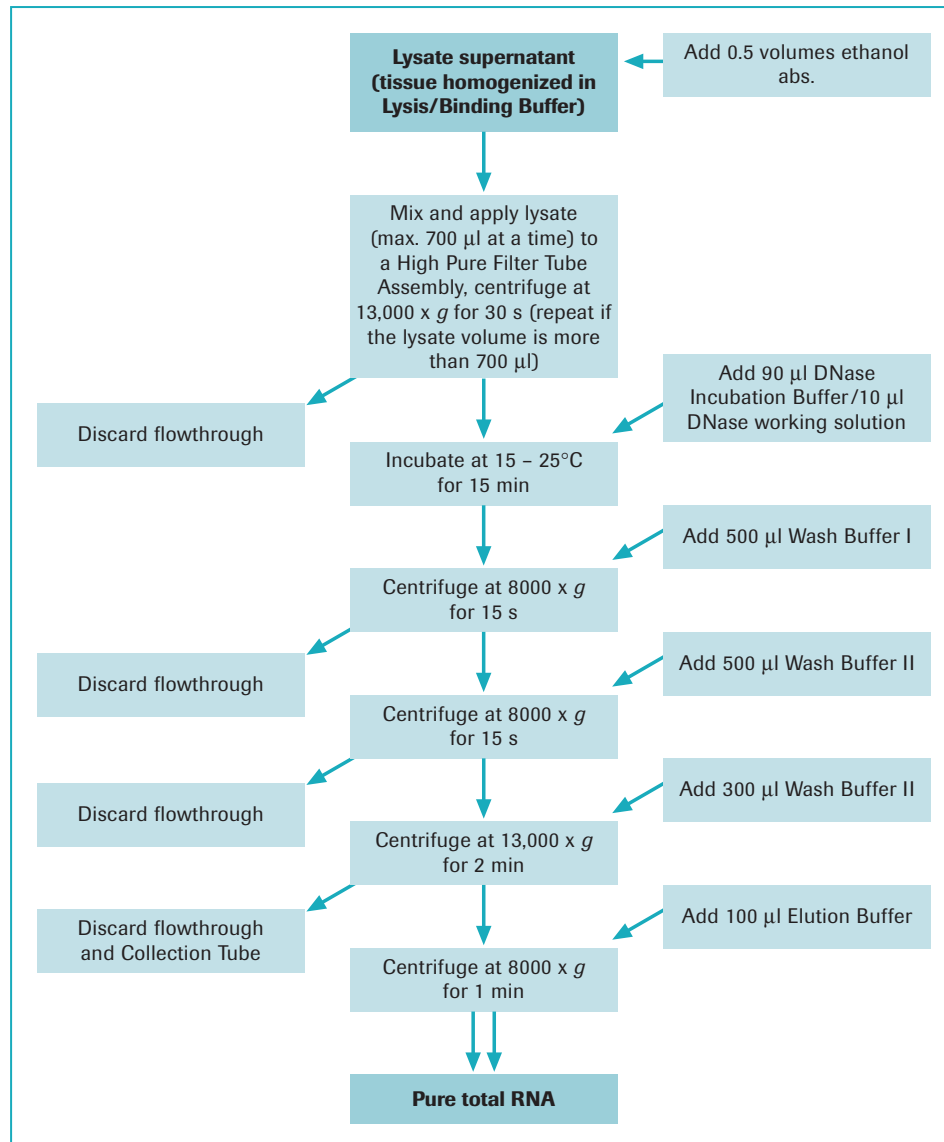
for up to 50 isolations of total RNA from tissue

Cat. No. 12 033 674 001

Principle	<p>Isolating intact RNA is a prerequisite for the analysis of gene expression. Frequently applied techniques like Reverse Transcriptase-PCR (RT-PCR), Northern blotting, and RNase protection require the use of intact undegraded RNA. Tissue samples are disrupted and homogenized in the presence of a strong denaturing buffer containing guanidine hydrochloride to instantaneously inactivate RNases, and to ensure isolation of intact RNA. After adding ethanol RNA binds selectively to a glass fiber fleece in the presence of a chaotropic salt (guanidine HCl). Residual contaminating DNA is digested by DNase I, applied directly on the glass fiber fleece. During a series of rapid "wash-and-spin" steps to remove contaminating cellular components the RNA remains bound to the glass fiber fleece. Finally, low salt elution removes the nucleic acids from the glass fiber. The process does not require RNA precipitation, organic solvent extractions, or extensive handling of the RNA.</p>
Starting material	<ul style="list-style-type: none"> ● Tissue (research samples, <i>e.g.</i>, mouse liver, spleen, lung, heart) ● 1 – 10 mg (for mortar/pestle disruption) or ● 1 – 25 mg (for rotor-stator homogenization)
Application	<ul style="list-style-type: none"> ● The kit prepares intact total RNA from tissue samples for direct use as template for RT-PCR or Northern blotting.
Time required	<ul style="list-style-type: none"> ● Total time: approx. 30 min with rotor-stator homogenization. Add additional time when using alternative disruption methods.
Results	<ul style="list-style-type: none"> ● Yield: 0.3 – 3 µg total RNA per mg tissue depending on tissue type. ● Purified RNA is free of DNA, nucleases and all cellular and sample components that interfere with RT-PCR. ● The absence of contaminating DNA is examined by a PCR without a preceding RT-reaction; no amplification product is obtained.
Benefits	<ul style="list-style-type: none"> ● Toxic materials are avoided, no CsCl gradient centrifugation and no extraction with organic solvents is necessary ● Eluates are ready to use, no alcohol precipitation has to be done

How to use the kit

I. Flow diagram



II. Kit contents

- Lysis/Binding Buffer (25 ml)
- DNase I, lyophilizate (10 kU)
- DNase Incubation Buffer (10 ml)
- Wash Buffer I (33 ml)
 - ⚠ Add 20 ml absolute ethanol before use
- Wash Buffer II (10 ml)
 - ⚠ Add 40 ml absolute ethanol before use
- Elution Buffer (30 ml)
- High Pure Filter Tubes, (50 tubes)
- Collection Tubes, 2 ml (50 tubes)

III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Mortar and pestle or Rotor-Stator Homogenizer (e.g., Ultra Turrax)

IV. Typical RNA yield from different type of mouse tissue

The yield of total RNA depends on the starting material and varies depending on the amount of tissue used and the kind of disruption method applied. The yield with mouse muscle tissue could not be determined spectroscopically, but isolated RNA resulted in a specific RT-PCR signal.

type of mouse tissue	Yield [$\mu\text{g}/\text{mg}$]
liver	0.5 – 2.8
kidney	0.5 – 1.0
spleen	0.5 – 3.0
lung	0.3 – 0.5
heart	0.3
muscle	n.d.

V. Isolation procedure

After you have prepared the working solutions and performed the disruption and homogenization of starting material you can start with the isolation procedure.

Standard reaction

- 1 Depending on the disruption and homogenization method, add one of the following to a nuclease-free 1.5 ml micro-centrifuge tube:
 - ▶ Add 400 μl Lysis/Binding Buffer and the appropriate amount of frozen tissue (max. 20 – 25 mg); disrupt and homogenize the tissue using a rotor-stator homogenizer.

alternative:

- ▶ Add 400 μl Lysis/Binding Buffer and the appropriate amount of tissue-powder (grinded with a mortar and pestle) and pass this lysate 5 – 10 times through a 20-gauge needle fitted to a syringe. For optimal yield do not exceed 10 mg tissue.

- 2 Centrifuge lysate for 2 min at maximum speed in a microcentrifuge and use only the collected supernatant for subsequent steps.
- 3 Add 200 μl absolute ethanol to the lysate supernatant and mix well.
- 4 Combine the High Pure Filter Tube and the Collection Tube and pipet the entire sample in the upper reservoir.
- 5 Centrifuge for 30 s at maximal speed (13,000 x g) in a standard table top microcentrifuge.
 - ! After this centrifugation step, the glass fleece must be dry; if it looks wet, the centrifugation time must be increased.

Discard the flowthrough and reassemble the Filter Tube and the used Collection Tube.

- 6 Into a sterile 1.5 ml reaction tube, pipet 90 μl DNase Incubation Buffer, add 10 μl DNase I working solution, mix. Pipet the solution in the upper reservoir of the Filter Tube. Incubate for 15 min at 15 to 25°C.



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- 7 Add 500 µl Wash Buffer I to the upper reservoir, and centrifuge for 15 s at 8000 x g.
- ▶ Discard the flowthrough and again reassemble the Filter Tube and the used Collection Tube.
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- 8 Add 500 µl Wash Buffer II to the upper reservoir, and centrifuge 15 s at 8000 x g. Discard the flowthrough and again reassemble the Filter Tube and the used Collection Tube.
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- 9 Add 300 µl Wash Buffer II to the upper reservoir of the Filter Tube, and centrifuge for 2 min at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.
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- 10 Carefully remove the column from the Collection Tube so that the column does not contact the flowthrough as this will result in carryover of ethanol.
- ⚠ *Residual ethanol may interfere with subsequent reactions.*
 - ▶ Discard the Collection Tube.
 - ▶ Insert the Filter Tube in a nuclease-free 1.5 ml reaction tube.
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- 11 Add 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
- ▶ Centrifuge 1 min at 8000 x g.
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- 12 The microcentrifuge tube now contains the eluted RNA.
- Either use 10 µl of the eluted RNA directly in RT-PCR or store the RNA at -80°C for later analysis.
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VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure Kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure Kits on page 98 of this manual. For factors that may affect the High Pure RNA Tissue Kit, see page 103.

Typical results with the kit

Comparison of different lysis/homogenization procedures

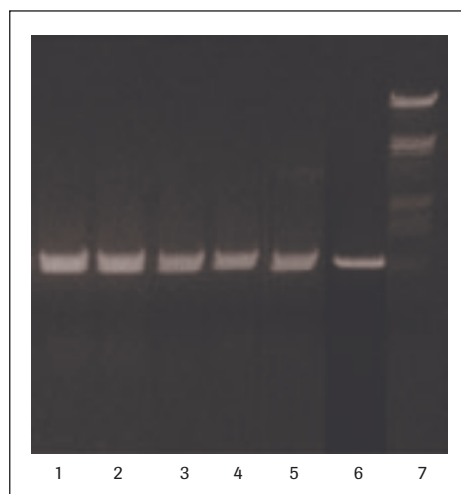


Figure 17: Mouse liver was homogenized with various procedures and RNA purified using the standard protocol of the High Pure RNA Tissue Kit. RT was performed with primers for a region of the GADH mRNA.

Lane 1: Ultra Turrax;

Yield: 1.9 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 2: Disposable plastic pestle, motor driven;

Yield: 3.0 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 3: Mortar + pestle/20G needle;

Yield: 1.5 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 4: Disposable plastic pestle-manual; 20 G needle;

Yield: 1.8 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 5: Disposable plastic pestle-manual;

Yield: 3.4 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 6: Bead-Vortex homogenization;

Yield: 3.0 µg/mg tissue; $OD_{260}/OD_{280} = 2.0$

Lane 7: MWM

Reference

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