


High Pure FFPE RNA Micro Kit

for isolation of total RNA from formalin-fixed, paraffin-embedded tissue samples

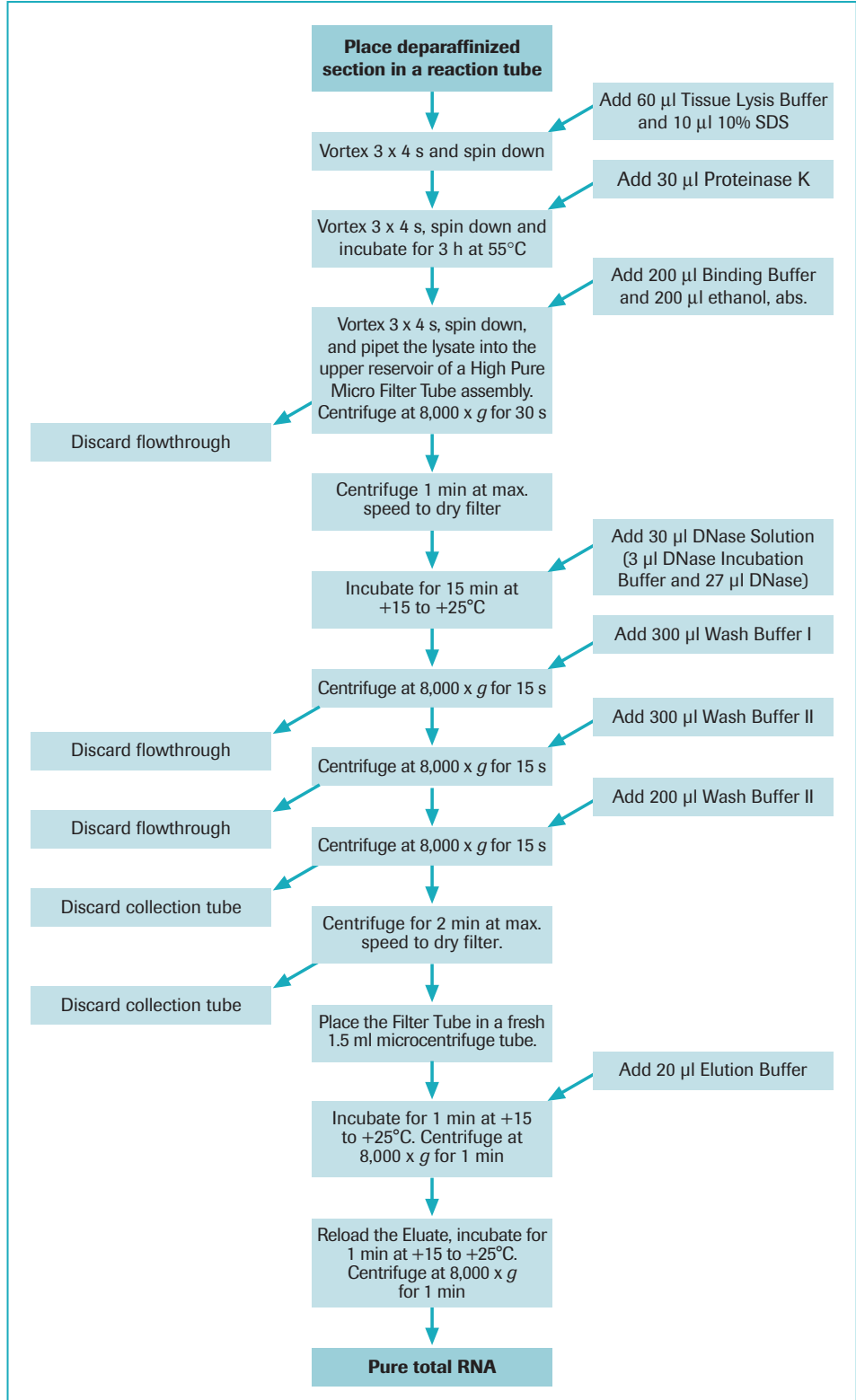
Cat. No. 04 823 125 001 (up to 50 isolations)

2

Principle	<p>To prepare tissue sections for RNA isolation, fixation reagents must be removed from the samples; after deparaffinization, the sections are ready to be processed with the High Pure FFPE RNA Micro Kit.</p> <p>The deparaffinized tissue samples are disrupted and homogenized during incubation with Proteinase K and chaotropic salt. The homogenate is then applied to the glass fiber fleece in a High Pure Micro Filter Tube.</p> <p>Under the buffer conditions used in the procedure, all nucleic acids (NA) bind specifically to the glass fleece in the High Pure tube, while contaminating substances (salts, proteins, and other tissue contaminants) do not. DNA is removed from the filter by digestion with DNase I. A series of rapid “wash-and-spin” steps remove the DNA fragments, along with other contaminating substances. Finally, the purified RNA is eluted from the column in low-salt buffer.</p>
Starting material	<ul style="list-style-type: none"> ● Amount: 1 – 10 µm sections ● Type: formalin-fixed, paraffin-embedded (FFPE) tissue ● Source: typical FFPE mammalian tissue (e.g., from colon, breast, liver, kidney, or spleen of mammal species incl. human research samples) <p> <i>Section thickness as well as yield and quality of the isolated RNA are strongly related to type of tissue, age of sample as well as fixation protocol used.</i></p>
Application	<ul style="list-style-type: none"> ● The High Pure FFPE RNA Micro Kit is designed for the isolation of total RNA from formalin-fixed, paraffin-embedded tissue samples for use in RT-PCR. The quality of RNA from paraffin-sections achieved with the kit is suitable for the relative quantification of mRNA with RT-PCR especially on the LightCycler® 2.0 System.
Time required	<ul style="list-style-type: none"> ● Total time required is approx. 60 min (without Proteinase K incubation)
Results	<ul style="list-style-type: none"> ● The High Pure FFPE RNA Micro Kit is optimal for FFPE sample material as shown by the high recovery of even small RNA fragments. Average yields are 1.5 to 3.5 µg / 5 µm section DNA-free RNA.
Benefits	<ul style="list-style-type: none"> ● Saves time with a simple and rapid protocol ● Obtain a highly concentrated eluate (10 µl) and recovery (>80%). ● Isolate DNA-free RNA, ideal for use in qualitative and relative quantitative RT-PCR ● Efficiently isolate RNA –even small RNA fragments. ● Generate high-quality template RNA that show excellent performance and linearity in RT-PCR. ● Rely on the innovative column design to efficiently recover contamination-free RNA.

How to use the kit

I. Flow diagram



II. Kit contents

- Tissue Lysis Buffer, 20 ml
- Proteinase K (recombinant, PCR grade), lyoph., 100 mg
- Binding Buffer, 80 ml
- Wash Buffer I, 33 ml
- Wash Buffer II, 10 ml
- DNase I, lyoph., 4 kU
- DNase Incubation Buffer, 10x conc., 1 ml
- Elution Buffer, 30 ml
- High Pure Micro Filter Tubes (containing glass fiber fleece), 5 x 10 tubes
- Collection Tubes, 2 x 50 tubes

III. Additional materials needed

- Absolute ethanol
- Ethanol 70%
- Hemo-De or Xylene
- SDS 10%
- Microcentrifuge tubes, 1.5 ml/2.0 ml, sterile
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal forces

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

IVa. Deparaffinization procedure for one 1-10 µm section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube.

- 1 To one 1 – 10 µm section in a 1.5 ml reaction tube add 800 µl Hemo-De (or Xylene), at least vortex for 4 s three times, incubate for 2 min, vortex at least for 4 s three times again and incubate for 5 min. Centrifuge for 2 min at maximum speed (12,000 – 14,000 x g) and discard supernatant by aspiration.
 - ! After this step directly cap the tubes to avoid tissue sections from drying.
 - ! If the tissue appears to be floating in the Xylene, spin for a further 2 min.

- 2 Repeat Step 1.

- 3 Add 800 µl ethanol abs., flick the tube to dislodge the pellet and vortex for 4 s three times. Centrifuge for 2 min at maximum speed (12,000 – 14,000 x g) and discard supernatant by aspiration.

- 4 Add 800 µl 70% ethanol flick the tube to dislodge the pellet and vortex for 4 s three times. Centrifuge for 2 min at maximum speed and discard supernatant by aspiration.

- 5 After removal of 70% ethanol, respin the tube for 10 – 20 s and carefully remove the residual fluid with a fine bore pipet. Incubate the open tubes in a heating block for 5 – 15 min at 55°C to air dry the tissue pellet. Proceed with step 1 of the RNA isolation protocol (IVc)

IVb. Deparaffinization procedure for one 1 – 10 µm section of formalin-fixed, paraffin-embedded tissue on a microscope slide.

- 1 Place the slide in a Hemo-De (or Xylene) bath and incubate for 10 min.
- 2 Tap off excess liquid and place the slide into ethanol abs. for 10 min.
- 3 Change bath and incubate the slide for a further 10 min in ethanol abs.
- 4 Scratch the deparaffinized section from the slide by using a sterile single-use scalpel and place it in 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 min at 55°C.
 - ▶ Proceed with step 1 of the RNA isolation protocol(IVc).

IVc. RNA Isolation Protocol from a 1-10 µm section of formalin-fixed, paraffin-embedded tissue.

! *If necessary, multiple preparations can be pooled after step 4.*

- 1
 - ▶ To one tissue pellet (deparaffinized as described above) add 60 µl Tissue Lysis Buffer and 10 µl 10% SDS
 - ▶ Vortex 3 x 4 s, spin down and add 30 µl Proteinase K working solution.
 - ▶ Vortex 3 x 4 s, spin down and incubate for 3 h at 55°C.
- 2 Add 200 µl Binding Buffer and 200 µl ethanol abs.
 - ▶ Vortex 3 x 4 s and spin down
- 3 Combine the High Pure filter tube with a collection tube and pipet the lysate into the upper reservoir.
- 4 Centrifuge for 30 s at 8,000 x g in a microcentrifuge and discard the flowthrough.
 - ! *Steps 3-4 can be repeated, in order to load the column with additional sample material (do not overload the column).*
- 5 Centrifuge for 1 min at max. speed to dry filter.
- 6
 - ▶ Add 30 µl DNase Solution (3 µl DNase Incubation Buffer and 27 µl DNase), Incubate for 15 min at +15 to +25°C.
- 7
 - ▶ Add 300 µl Wash Buffer I working solution to the upper reservoir.
 - ▶ Centrifuge for 15 s at 8000 x g, discard the flowthrough.
- 8
 - ▶ Add 300 µl Wash Buffer II working solution
 - ▶ Centrifuge for 15 s at 8000 x g, discard the flowthrough.
- 9 Add 200 µl Wash Buffer II working solution centrifuge for 15 s at 8000 x g, discard the collection tube.
- 10 Place the High Pure Micro filter tube in a fresh collection tube and centrifuge for 2 min at maximum speed.
- 11
 - ▶ Place the High Pure Micro filter tube in a fresh 1.5 ml reaction tube, add 20 µl Elution Buffer and incubate for 1 min at +15 to +25°C.
 - ▶ Centrifuge for 1 min at 8000 x g.
- 12
 - ▶ Reload the eluate; incubate for 1 min at +15 to +25°C. Centrifuge at 8000 x g for 1 min



- 13 The microcentrifuge tube now contains the eluted RNA. Either use 0.5 – 5 μ l of the eluted RNA directly in RT-PCR or store the eluted RNA at -80°C for later analysis.
- ! Before photometric determination of the RNA concentration, centrifuge the eluate for 2 min 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.

V. 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 protocols, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure FFPE RNA Micro Kit, see page 104.

Typical results with the kit

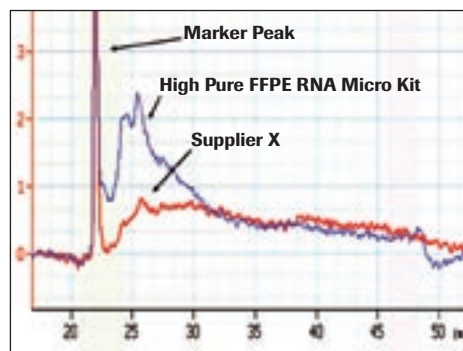


Figure 18: Fragment-length distribution of isolated RNA. RNA was isolated from a 5 μ m section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The size distribution of the recovered RNA fragments was determined on an electropherogram (Bioanalyzer, Agilent).

Result: The High Pure FFPE RNA Micro Kit recovers optimal amounts of the different RNA fragments in the sample, even the small ones.

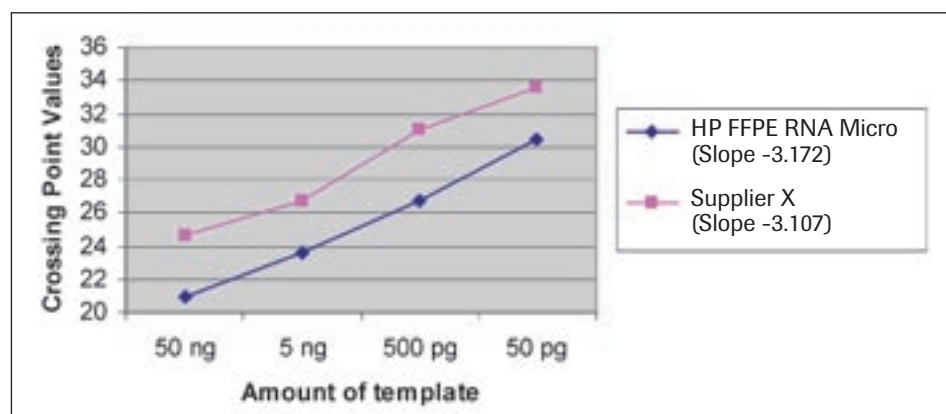


Figure 19: Performance of isolated RNA in RT-PCR. RNA was isolated from a 5 μ m section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The isolated RNA samples were serially diluted and used as templates in separate RT-PCRs. A β -2microtubulin-specific amplification was performed, using the LightCycler[®] 1.5 Instrument and LightCycler[®] RNA Amplification Kit SYBR Green I.

Result: Template RNA isolated with the High Pure FFPE RNA Micro Kit performs well in the RT-PCR, giving linear results (based on the consistent slope obtained with serial dilutions) and high sensitivity (based on the early crossing points observed).

References

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