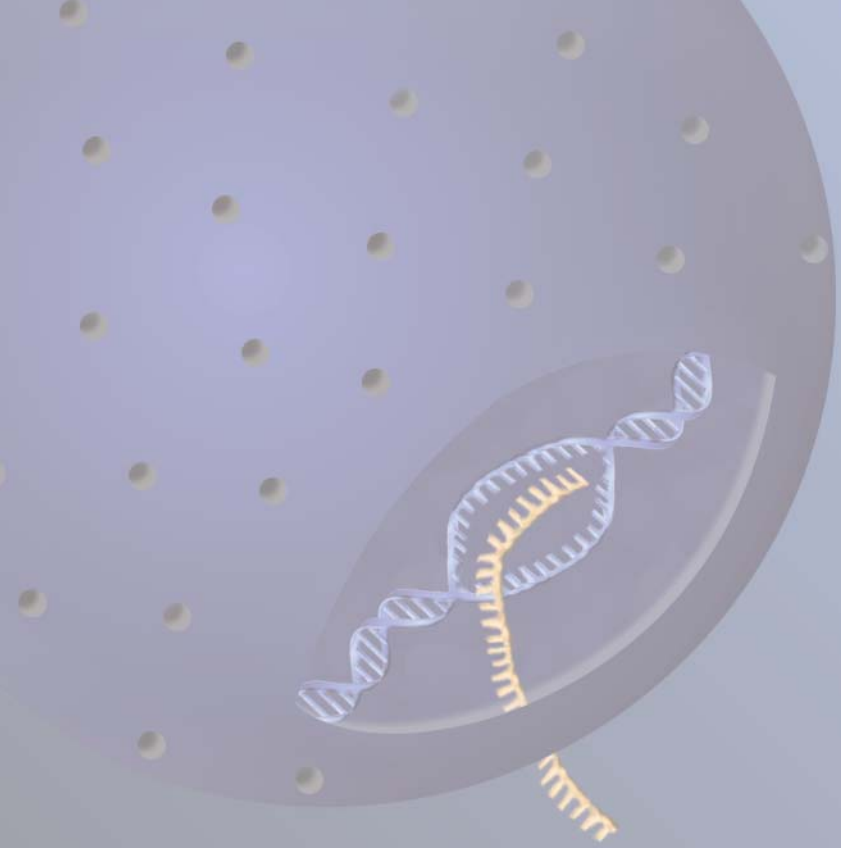

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RNA extraction from FFPE sections

ZEISS

We make it visible.

Some helpful tips before starting:

- To find additional information around RNA work - especially for less experienced users - please consult: „**PALM Protocols - RNA Handling**“ which is available on request.

- We recommend MembraneSlide1.0 PEN (Order No. 415101-4401-000) for routine preparation of FFPE sections. If weak fluorescence must be detected or ablation is needed before micro-dissection MembraneSlide 1.0 PET (Order No. 415101-4401-050) may be applicable.
  For other special applications please inquire.

- To ensure RNase-free conditions use only RNase-free solutions and materials.
  Incubate MembraneSlides in dry heat at 180°C for 4 hours to completely inactivate any RNases.

- Microtome cutting, transfer, deparaffination and staining can be done according to standard procedures. Longer melting/drying of the section improves the adhesion to the membrane.

- Most standard histological stains (like HE, Methyl Green, Cresyl Violet, Nuclear Fast Red) can be used for FFPE material. To our experience only Methylene Blue is not recommendable for RNA.

Note: To allow cutting and lifting a *coverslip* and mounting medium *must not be applied*!

- For collecting microdissected samples we recommend the special AdhesiveCaps:
  AdhesiveCap 500 opaque (Order No. 415101-4400-250) or
  AdhesiveCap 500 clear (Order No. 415101-4400-255)

- The incubation with Proteinase K in the PALM protocol is prolonged significantly compared to the original QIAGEN manual because all our tests with laser microdissected material from various tissues showed better RNA yields by applying longer digestion times.

Note: For formalin fixed samples a **Proteinase K** digestion step is essential. The time necessary for optimal Proteinase K digestion depends on many factors like tissue type, fixation procedure or element size of lifted material. An overnight digestion (12-18 hours) is a good starting point for optimization, but shorter digestion times may be tested as well. To our experience digestion of at least 3 hours should be applied with any extraction procedure and FFPE material.

- We normally use 5 to 10 µl of the final RNA solution in a RT-reaction of 20 µl (e.g., Transcriptor First Strand cDNA Synthesis Kit, ROCHE, # 04 379 012 001) using random-oligomers (instead of oligoT) as primers for the cDNA synthesis.

Note: The use of **random or gene-specific primers** is very important since reverse transcription of formalin fixed RNA with only standard oligoT-primers will be very inefficient and strongly 3`biased due to the numerous strand breaks and modifications inflicted by the formalin fixation and paraffin embedding procedure.

- To prognose the extractable amount of RNA from FFPE-tissue is very difficult since many factors like species, cell/tissue-type, fixation, staining, fragmentation, modification and others will strongly influence the outcome. Any FFPE-tissue block should therefore be tested individually.

Using components of the QIAGEN RNeasy® FFPE Kit-

1. Add 150 µl Buffer PKD and 10 µl of Proteinase K to the tube, containing the LCM elements in the AdhesiveCap, and vortex in an "upside down" position.
2. Use an incubator to digest the samples in an "upside down" position at 55°C overnight (or for at least 3 hours), then vortex and heat at 80°C for 15 min in a heating block.
3. Add 320 µl of Buffer RBC to adjust binding conditions.
4. Mix the lysate thoroughly and transfer it to a gDNA Eliminator spin column placed in a 2 ml collection tube. Centrifuge for 30 sec at ≥8000 x g (e.g. Eppendorf 5415D: ≥10000 rpm).
   Discard the column and save the flow-through.
5. Add 720 µl of 100% ethanol to the flow-through and mix well by pipetting. Do not centrifuge. Proceed immediately to the next step.
6. Transfer 700 µl of the sample to a RNeasy MinElute spin column placed in a 2 ml collection tube.
   Close the lid gently and centrifuge for 15 sec at ≥8000 x g (≥10000 rpm). Discard the flow-through. Reuse the collection tube in step 7.
7. Repeat step 6 until the entire sample has passed through the RNeasy MinElute spin column. Reuse the collection tube in step 8.
8. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently and centrifuge for 15 sec at ≥8000 x g (≥10000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 9.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.
9. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently and centrifuge for 15 sec at ≥8000 x g (≥10000 rpm) to wash the spin column. After centrifugation carefully remove the spin column from the collection tube so that the column does not contact the flow-through.
10. Place the RNeasy MinElute spin column in a new 2 ml collection tube, and discard the old collection tube with the flow-through. Open the lid of the spin column and centrifuge at full speed for 5 min.
    Discard the collection tube with the flow-through. It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions.
11. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube.
    Add 14-30 µl RNase-free water directly to the spin column membrane.
    Close the lid gently and centrifuge for 1 min at full speed to elute the RNA.
    The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl of RNase-free water results in a 12 µl eluate.
12. The RNA solution may be stored at -20°C or used directly for reverse transcription.

    Note: Quality control by direct analysis like the Agilent Bioanalyzer (Pico chip) is very limited and may only be possible with quite large microdissected samples (often some 4 mm^2 collected area from tissue sections of 5-10 µm thickness).

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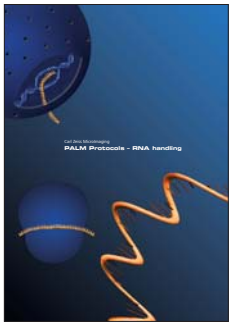
PALM Laboratories recommend the **QIAGEN RNeasy® FFPE Kit** (#74404) to isolate RNA from **F**ormalin **F**ixed, **P**araffin **E**mbedded tissue. But some PALM specific modifications to the protocol are necessary.

The RNeasy®-procedure is very efficient and allows a high final concentration of RNA due to a small elution volume (12 µl). Genomic DNA contamination is minimized by a special DNA removal column (gDNA Eliminator spin column).

Protocols on request:



PALM Protocols RNA handling

Short Protocol RNA extraction from frozen sections

Short Protocol RNA extraction from FFPE sections

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