ZEISS Microscopy Labs

Scientific Support, Training and LabService

RNA extraction from FFPE sections

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Some helpful tips before starting:

- To find additional information around RNA work - especially for less experienced users - please consult: "**LCM Protocols - RNA Handling**" which is available at www.zeiss.de/applab.

- We recommend MembraneSlide1.0 PEN (Order No. 415190-9041-000) 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. 415190-9051-000) 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. We recommend incubation at 56°C over night (≥ 16 h) before deparaffination.

- 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 laser cutting and lifting a *coverslip* and mounting medium *must not be applied*!

- For collecting microdissected samples we recommend special AdhesiveCaps:
  AdhesiveCap 200 opaque (Order No. 415190-9181-000) or
  AdhesiveCap 200 clear (Order No. 415190-9191-000)

- The incubation with Proteinase K in our 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 any formalin fixed material should be digested for at least 3 hours, no matter which extraction procedure is used.

- 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 oligo dT) 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 oligo dT-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- (Order No. 73504)

1. Add 150 µl Buffer PKD and 10 µl of Proteinase K to the tube containing the LCM elements in the AdhesiveCap and invert the tube to get contact between liquid and adhesive surface.
2. Use an incubator to digest the samples in an "upside down" position at 56°C overnight (or for at least 3 hours), then vortex and heat at 80°C for precisely 15 min in a heating block.

   Note: Please do not use any water bath for the upside down incubation.
3. Incubate on ice for 3 min.
4. Add 16 µl DNase Booster Buffer and 10 µl DNase I stock solution. Mix gently by inverting the tube. Centrifuge briefly to collect residual liquid from the sides of the tube.

   Note: DNase I is supplied lyophilized and should be reconstituted as described in "Preparing DNase I stock solution" (page 14; RNeasy FFPE handbook 09/2010).

   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex!
5. Incubate at room temperature for 15 min.
6. Transfer the lysate to a new 1.5 ml microcentrifuge tube.
7. Add 320 µl Buffer RBC to adjust binding conditions and mix the lysate thoroughly.
8. Add 720 µl ethanol (100%) to the sample and mix well by pipetting. Do not centrifuge. Proceed immediately to step 9.
9. 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 10.
10. Repeat step 9 until the entire sample has passed through the RNeasy MinElute spin column. Reuse the collection tube in step 11.
11. 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 12.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.
12. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently and centrifuge for 2 min 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.
13. 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.
14. 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.
15. The RNA solution may be stored at -20°C or used directly for reverse transcription.

    Note: Quality control by direct analysis (e.g. Agilent Bioanalyzer Pico Chip) is very

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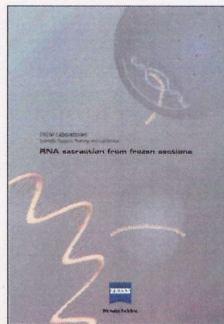
ZEISS Microscopy Labs recommend the **QIAGEN RNeasy® FFPE Kit** (#73504) to isolate RNA from **F**ormalin **F**ixed, **P**araffin **E**mbedded tissue.
But some LCM specific modifications to the protocol are necessary.

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 DNase I digest on the purification column.

Protocols on request:

LCM Protocols
RNA handling



Short Protocol
RNA extraction
from frozen sections

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